UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
F. EDWARD HÉBERT SCHOOL OF MEDICINE
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

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Name of Candidate:   Joshua Tomchesson
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Dissertation and Abstract Approved:

Jerome Singer, Ph.D.                                       31 March 2006
Department of Medical & Clinical Psychology               Date
Committee Chairperson

Neil Grunberg, Ph.D.                                       31 March 2006
Department of Medical & Clinical Psychology               Date
Committee Member

Tracy Sbrocco, Ph.D.                                       31 March 2006
Department of Medical & Clinical Psychology               Date
Committee Member

Mazen Saah, Ph.D.                                          3-31-06
Department of Medical & Clinical Psychology               Date
Committee Member

Joseph McCabe, Ph.D.                                       3-31-06
Department of Anatomy, Physiology & Genetics              Date
Committee Member

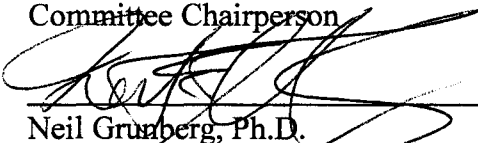The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

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Joshua L. Tomchesson
Department of Medical and Clinical Psychology
Uniformed Services University of the Health Sciences

# ABSTRACT

Title of Thesis:    "Effects of Environmental Conditions on Activity, Feeding, and Body Weight in Male and Female Adolescent Rats"

Author:    Joshua L. Tomchesson, Doctor of Philosophy, 2006

Thesis directed by:  Neil E. Grunberg, Ph.D.

Professor

Department of Medical and Clinical Psychology

Excessive body weight, particularly in children, is a growing concern in the United States and around the world.  Body weight is affected by feeding behavior and physical activity.  Environmental factors affect feeding behaviors and physical activity; therefore, environment is an important influence on body weight. Three separate experiments examined the behavioral effects of environmental enrichments on feeding, activity, and body weight.  For the first two experiments, subjects were 36 adolescent, male (Experiment I) and 36 adolescent female (Experiment II) Sprague-Dawley rats.  Experiment III examined the behavioral effects of enrichment on 24 male and 24 female adolescent Sprague-Dawley rats.  Responses to environmental enrichment included: body weight (BW), Body Mass Index score (BMI), Lee Index score (LI), consumption of standard rat chow, Oreo$^{TM}$ cookies, and Lays$^{TM}$ potato chips, and physical activity (PA) in the animal's home cage (HCA) and in an open field (OF).

The major findings from these experiments were that: 1) environmental enrichment results in lower body weight, 2) environmental enrichment decreased

food consumption, especially the bland foods, 3) animals housed in environmental enrichment were less active in novel surroundings and were more active in their home cages compared to animals in non-enriched housing, and 4) males and females responded similarly to environmental enrichment with regard to body weight, feeding, and physical activity. These findings highlight the importance of the effects of housing conditions in animal research and suggest ways to help control body weight in animals and humans.

Effects of Environmental Conditions on Activity, Feeding, and Body Weight in

Male and Female Adolescent Rats

by

Joshua L. Tomchesson

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*"In order to attain the impossible, one must attempt the absurd."*
**- Miguel de Cervantes**

In the beginning, the endeavor to become a skillful clinician and scientist appeared impossible. With desire, determination, the support of family and friends, and guidance from some extraordinary mentors, we attempted the absurd. What followed was an incredible experience unlike anything I ever imagined and the results of this expedition transcend what is written in this manuscript. This doctoral dissertation represents the successful completion of the first phase of my endeavor, a journey that does not seem impossible anymore. The successful completion of this phase of training would not have been possible without the knowledge, support, and guidance of many people.

First and foremost, I want to thank Jenn, my wife. Your unending love, optimistic spirit, and unwavering support made attempting the absurd possible. I am a better husband, father, and person because of you. With you, my life has meaning and purpose. Without you, I would be incomplete. To my children, Jared, Skye, and Jaxon, your sacrifices have not gone unnoticed. Thank you for your support and understanding when Dad had to work. You give me joy every minute of every day of my life. To my parents, Jessie, Ken, Terry, and Tony, your love, guidance, and encouragement have made me the man I am today.

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## SECTION I – INTRODUCTION

### Overview

Physical and social aspects of an environment can influence the biology and behavior of organisms. Biological changes in response to environmental manipulations can include changes in: brain structure (e.g., Rosenzweig, Bennett, & Diamond, 1972; Rosenzweig & Bennett, 1996; Mohammed et al., 2002), endogenous hormones such as corticosterone (Bhatnagar & Meaney, 1995; Mering Kaliste-Korhonen & Nevalainen, 2000; Belz, et. al., 2003), and body weight (Brown & Grunberg, 1995; O'Conner & Eikelboom, 2002; Hellemans, Benge, & Olmstead, 2004). Behavioral changes related to enriched environmental manipulations typically include improved performance on various tasks, such as maze performance and responses to novel situations. These improvements suggest that environmental enrichment improves learning, memory, and information processing (e.g., Hebb, 1947; Greenough & Juraska, 1979; Daniel, Roberts, & Dohanich, 1999; Varty et. al., 2000). The effects of environmental enrichment may be clinically relevant beyond learning, memory, or information processing changes because environmental changes also affect behaviors that are directly relevant to health. For example, environmental influences, such as cage size (Steyermark & Mueller, 2001), handling (Meaney, et al., 1992), type of available foods (Sclafani & Springer, 1976), and opportunities for activity (Lattanzio & Eikelboom, 2003), have been reported to affect food consumption and body weight of animals, two key factors of an organism's health.

This research project focused on the behavioral effects of environment on body weight because body weight is a current concern in our society. For example, approximately 65% of adults and 30% of children are at an increased risk for negative health related consequences associated with excessive body weight, such as premature death, diabetes mellitus, hypertension, cardiovascular disease, and certain kinds of cancers (Pi-Sunyer, 2004). This doctoral research included three experiments that were conducted to examine the extent to which: (1) environmental enrichment affects body weight, (2) environmental enrichment alters consumption of a variety of foods, (3) environmental enrichment affects physical activity, and (4) gender differences exist in these effects in adolescent rats. As background for this research, Section I reviews the relevant literature on environmental enrichment and body weight. Section II presents an overview for the work, the rationale for each independent and dependent variable, a description of the research design, and the data analytic strategy. Next, the hypotheses, methods, results, and a brief discussion for each of the three experiments are presented. Section III summarizes and discusses the findings of the project as a whole, including the limitations of this project and future directions for this research. Section IV includes relevant Tables, Figures, and References.

## Enriched Environments

The concept of environmental enrichment has been used to improve the lives of animals and humans. Environmental enrichment refers to providing opportunities for organisms to thrive and excel. For captive animals, enrichment

includes providing physical objects and the opportunities for social interaction to create more naturalistic environments compared to barren cages (Shepherdson, 1992; Rosenzweig & Bennett, 1996). For humans, environmental enrichment includes providing individuals opportunities to be creative, learn, develop new skills, and promote growth (e.g., good nutrition, supportive and loving environments, performing music, painting, learning new languages, playing sports) (Diamond, 1999).

Environmental enrichment has long-lasting positive biological and behavioral consequences such as increasing brain size, and improving information processing and learning (e.g., Premack & Premack, 1963; Rosenzweig, Bennett, & Diamond, 1972; Rosenzweig & Bennett, 1996; Hellemans, Benge, Olmstead, 2004). In contrast, animals reared without physical or social stimulation exhibit less learning, decreased memory, and disrupted information processing compared to enrichment-reared animals (e.g., Greenough & Juraska, 1979; Woodcock & Richardson, 2000). Environmental changes also affect behaviors that are directly relevant to health, such as physical activity (e.g., Premack & Premack, 1963; Boakes & Dwyer, 1997; Lattanzio & Eikelboom; 2003) and food consumption (e.g., Brown & Grunberg, 1995; O'Conner & Eikelboom, 2002).

Research on environmental enrichment suggests that stimulating environments are important for healthy development and may influence the long-term health of the animals housed in these environments. Section I provides a discussion of environmental enrichment, the primary independent variable in the

proposed research project. A brief historical perspective provides the context to present the biological and behavioral effects of environmental enrichment and discuss the application of animal enrichment research findings to humans.

### *History of Enriched Environments*

The notion of using the environment to affect future performance and behavior can be traced to early Chinese teachings. The concept of "intrauterine education" (i.e., educating a child during development within the womb) can be found in Chinese literature during the Ming Dynasty, around 1237 AD (Diamond & Hopson, 1999). Women were advised to behave favorably during pregnancy so that their offspring would be bright and live well. Favorable behavior included: sitting and walking in a dignified and sedate manner, maintaining a good temper with a mind at ease, not looking at evil happenings or ugly pictures (Diamond & Hopson, 1999). The popularity of intrauterine education is alive in our culture today, evidenced by products such as "Baby Bach" music CDs and concepts such as reading to the fetus through the mother's belly to facilitate fetal intellectual development. The current conceptualization of environmental enrichment originated with Charles Darwin (1872).

Darwin (1872) reported that the brains of domestic rabbits were considerably smaller compared to the brains of wild rabbits. He argued that the reduced brain size of the domestic animals was a consequence of a deprived environment. Specifically, domesticated animals lived in inactive environments and did not exert their intelligence, instincts, or senses as much as animals did in

the wild.  Empirical support for Darwin's observations and interpretation would not appear in the literature for decades.

In 1925, Robert Yerkes wrote about his experiences studying colonies of monkeys and apes in Cuba in his book Almost Human.  He wrote that companionship and play were as important to a primate's health, comfort, and contentment as were physical environment and factors such as temperature, moisture, food, and drink.  Further, he stated that no primate should be kept in isolation.

> "Undoubtedly, kindness to captive primates demands ample provision for amusement and entertainment as well as for exercise. The greatest possibility of improvement in our provision for captive primates lies in the invention and installation of apparatus which can be used for play or work." (Yerkes, 1925, p. 25)

Providing ample provisions for captive animals serves as a guiding principal for zoology and animal husbandry personnel throughout the world today.

In 1947, Donald Hebb observed that laboratory rats that he had taken home for his children to play with performed better on maze learning tasks when returned to the laboratory as compared to rats kept solely in the laboratory environment.  He concluded that nerve cells in the brains of the rats had changed in response to the enriched and varied experiences outside the laboratory.  Hebb hypothesized that the number of synaptic connections increased and that these structural changes resulted in functional (i.e., behavioral) modifications.  These changes were believed to reflect new learning.  Remarkably, Hebb's report,

which was consistent with Darwin's (1872) observation, did not generate research for almost 20 years.

Mark Rosenzweig and his colleagues at the University of California, Berkeley, introduced the classic paradigm of enriched environments in 1966. In Rosenzweig's (1966) enrichment, animals were provided both social and physical stimulation. Animals were housed in groups to provide opportunities for social interaction (i.e., social enrichment). Physical stimulation (i.e., physical enrichment) involved placing objects in the animals' cages to allow tactile stimulation and physical activity (Rosenzweig & Bennett, 1996; Woodcock & Richardson, 2000). Enriched environments are distinguished from non-enriched environments by the amount of stimulation and activity available in the environment. The standard non-enriched environment limits the physical and social enrichment by housing the animals individually without objects (Varty, Paulus, Braff, & Geyer, 2000). Most current environmental enrichment studies include both social and physical enrichment components (e.g., Pham et al., 1999; Passineau, Green, & Detrich, 2001; Tomchesson, 2004).

There is no one method of environmental enrichment in the research literature. There are many different ways to manipulate and conceptualize environmental enrichment in animals including: neonatal handling (Meaney, Aitken, Sharma, & Viau, 1992), pretest handling (Schmitt & Hiemke, 1997), social enrichment (Renner & Rosenzweig, 1986; Varty et al., 2000), physical enrichment (Renner & Rosenzweig, 1986; Varty et al., 2000), and incorporation of natural environmental objects (Schrijver et al., 2002). Enriched environments

also vary in the amount of time animals are exposed to enrichment ranging from 12 days (Passineau, Green, & Detrich, 2001; Elliott & Grunberg, 2005) to a year (Ickes et al., 2000). The most common enriched environments in animal research house 3 to12 subjects per cage filled with toys and objects (e.g., pieces of wood, plastic bones, exercise wheels, balls, tunnels) for a minimum of 2 - 4 weeks.

### *Effects of Enriched Environments*

Enriched housing environments have different biological and behavioral consequences when compared to non-enriched housing. Although this research project focused on the behavioral consequences, biological consequences are also important to this research. This section briefly reviews biological and behavioral consequences of environmental enrichment (EE).

### *Biological Effects of Enrichment*

Although human beings are born with 100 billion neurons that are surrounded by over one trillion glial cells that protect and nourish these neurons, the pattern of "wiring" necessary for communication between the cells is not yet stabilized at birth (Nash, 1997; Joseph, 1999). For example, the number of synapses in one layer of the visual cortex increases from approximately 2,500 connections at birth to as many as 18,000 connections only six months later (Kliem et al., 1998). The environment may contribute to the exact wiring that occurs because altering environments appears to alter brain cytoarchitecture (Mohammed et al., 1993; Rosenzweig, 1996; Diamond, 2001).

Animal experiments reveal that physical and social stimulation (i.e., environmental enrichment) evoke the same cascade of neurochemical events that cause plasticity alterations in the human brain (Rosenzweig & Bennett, 1996). Stimulating environmental conditions (i.e., enriched environments) significantly influence brain development. These influences include: increased size and weight of the cortex, increased neuron sizes and dendritic branching, increased synapse formation, and elevated protein levels (Rosenzweig, Bennett, & Diamond, 1972; Mohammed et al., 2002). Diamond (1991) reported that laboratory rats housed in enriched environments could have up to 25 percent more neurons in their brains when compared to rats housed in non-enriched environments. In addition to biological changes in animals, behavioral changes also have been reported.

***Behavioral Effects of Enrichment***

In addition to the increased number of neurons in the brain, rats reared in an enriched environment exhibit more complex behaviors than rats reared in non-enriched environments (Haywood & Tapp, 1966; Mohammad et al., 1993; Pham et al., 1999; Kobayashi, Ohashi, & Ando, 2002). Environmental enrichment can significantly improve the cognitive functioning of animals. This improvement is inferred from the animal's performance on behavioral tasks of attention, memory, and learning compared to animals reared in standard non-enriched environments. For example, early social isolation leads to an interruption of attentional processing in rats as measured by acoustic startle reflex (Robbins, 1996). Rats reared with environmental enrichment are better

able to identify relevant environmental cues that decrease the magnitude of their reflexive responses to loud noises. Also, rats deprived of social contact post-weaning (i.e., when social play normally develops) have an impaired ability to process information as measured by pre-pulse inhibition (PPI) of the acoustic startle reflex, believed to index an innate sensorimotor "gating" mechanism that underlies the organism's ability to select relevant stimuli from the environment while screening out irrelevant information (Swerdlow, Caine, Braff, & Geyer, 1992).

Superior learning and memory task performance by rats reared in enriched environments is well documented (Greenough & Juraska, 1979). Woodcock and Richardson (2000) reported superior information processing and working memory for rats raised in enriched environments compared to rats raised in non-enriched environments. Rats reared in enriched environments were better able to discriminate between two types of cages: a conditioning cage used to train the animals and a similar cage that looked like the conditioning cage but was distinctly different. The Morris water maze and the radial maze tasks are widely used measures of rodent learning and spatial memory. When compared to rats housed in non-enrichment conditions, the rats housed in enriched conditions perform significantly better in the Morris water maze task (Daniel, Roberts, & Dohanich, 1999; Williams, Luo, Ward, Redd, & Gibson, 2001; Elliott, 2004) and the radial maze (Einon, 1980). Enriched housing environments also result in more rapid decreases in locomotor activity in novel environments, indicating faster learning and adaptation to the new environment (Elliott, 2004). Similarly,

rats reared in enriched environments display quicker adaptation of the acoustic startle response than rats reared in non-enriched environments (Swerdlow, Caine, Braff, & Geyer, 1992; Wilkinson et al., 1994; Elliott, 2004).  These findings suggest that environmental enrichment enhances the animal's ability to process information, thereby improving the overall cognitive abilities of rats housed in enriched environments compared to rats housed in non-enriched environments.

The benefits of environmental enrichment are not limited to rats and have been reported in other animals.  Two examples of the diverse species that have benefited from environmental enrichment are seals and pigs.  Grindrod and Cleaver (2001) reported that incorporating novel toys, such as rubber balls, and opportunities to forage for food reduced captive seals' stereotypic circling behavior.  Stereotypic behaviors (e.g., circling or pacing) are abnormal repetitive behaviors commonly thought to represent anxiety or stress in animals (Grindrod & Cleaver, 2001).  Consequently, the addition of toys and opportunities to work for food reduced the emotional response of the seals to their environment.  In addition to seals, pigs also benefit significantly from environmental enrichment. Enrichment for pigs consists of social housing, straw, and toys such as cloth strips, chains, dirt, and ropes (Grandin, Curtis, & Greenough, 1983; Grandin, Curtis, & Taylor, 1987).   Pigs housed in barren environments will use penmates as substitutes for other objects resulting in harmful social behaviors (Buré, 1981; Burbidge et al., 1994).  However, enlarged housing spaces and partial stalls used to keep the animals from seeing each other, decreases aggression, reduces cortisol concentrations, and increases immunological responsiveness (Andersen

et al., 1999).  Wemelsfelder, Haskell, Mendl, Calvert, and Lawrence (2000) suggest that enriching the environment of pigs counteracts frustration and boredom that typically results from chronic under-stimulation.  Additionally, pigs reared in enriched environments exhibit more diverse behaviors than pigs reared in non-enriched environments (Whittaker et. al., 1998; Wemelsfelder et al., 2000).

Enriched environments, characterized by the presence of physical objects and the opportunity for social interaction, have been shown to enhance healthy brain development and provide marked improvements in performance.  Research has demonstrated that enrichment leads to improved performance in learning tasks and a significant reduction of emotional responses to the environment.  In contrast, non-enriched environments or reduced sensory stimulation, such as social isolation and the lack of physical objects, results in performance deficits in learning tasks and expressed hyper-emotionality.  The benefit of environmental enrichment for animals is important in the context of captive environments; however, the power of environmental enrichment is in its application to humans.

### *Relevance of Enriched Environments to the Human Condition*

Developed from animal research, the concept of environmental enrichment has been used effectively by humans.  In the late 1960's, Mark Rosenzweig, Marion Diamond, and their colleagues reported neurological changes within the animal brain in response to environmental enrichment.  These changes include increased size and weight of the cortex, increased neuron sizes and dendritic branching, and increased synapse formation.  Based on these reports and

findings that environmental enrichment improved performance on behavioral learning tasks, the inference that the reported neurological changes accounted for the improved performance was extrapolated to humans. Consequently, the animal research on environmental enrichment inspired human enrichment programs to attempt to improve learning, memory, and information processing.

The application of environmental enrichment to help humans proved to be a valuable exercise. Research has shown that environmental stimulation appears to be necessary for healthy brain development and may affect behaviors later in life. For example, children raised in impoverished environments exhibit impairments in cognitive and behavioral functioning, whereas children raised in highly stimulating or enriched environments exhibit enhanced behavioral and cognitive outcomes (Kaler & Freeman, 1994; Joseph, 1999). Haywood and Tapp (1966) suggested that limited intelligence and social functioning often accompany "unstimulating" childhood conditions. Experimental enrichment programs have been able to offset some of the cognitive and social deficits created by unstimulating conditions. In a recent study, an environmentally-based intervention showed promising results in Portuguese elementary school children who were previously exposed to adverse environmental factors (e.g., malnutrition, familial distress, and low family income). Children were placed together in a boarding house (i.e., social enrichment) and were taught using a learning program that included psychomotor exercises, painting, singing, computer training, and language skills (English and Spanish). Placing the children in the social housing condition and enriching their instruction significantly

improved the children's school attendance and classroom behavior. More importantly, this better school attendance was correlated with significant improvements in the students' cognitive performance (Macedo, Andreucci, & Montelli, 2004). Therefore, the enrichment program resulted in improvements in behavior and academic performance.

One of the most notable applications of environmental enrichment is the introduction of early education programs, such as "Head Start." "Head Start" is a comprehensive preschool program that began in 1965. It was designed to meet the emotional, social, health, nutritional, educational, and psychological needs of children, ages 3 to 5, from low-income families. In general, the research on the efficacy of such early education programs indicates that participating in these programs improves cognitive abilities and skill attainment for children exposed to these enrichment programs, compared with children who were not exposed to early educational programs, for up to several years after the end of the program (Behrmen et al., 1995). Reynolds and colleagues (2001) completed a 15-year follow-up study that examined specific behavioral consequences of early education and reported significant differences between children who attended "Head Start" programs and those who did not. Children in "Head Start" programs had lower high school drop-out rates (46.7% vs. 55%), a lower rate and number of juvenile arrests (16.9% vs. 25.1%), lower rates of children retained in their current academic grade (23% vs. 38.4%), and lower rates of required special education programs (15.4% vs. 21.3%) (Reynolds et al., 2001).

Additional research suggests that environmental enrichment may produce behavioral changes in children, such as increased impulse control, more systematic planning and organization, and more academic risk-taking in the classroom (Wilson, 1996). There are consistent data suggesting that students who take music and art classes acquire higher SAT and College Board scores (The College Board, 1996-2004). These higher scores reflect improved cognitive abilities such as verbal skills, math, and abstract reasoning (Graziano, Peterson, & Shaw, 1999). Further, a positive correlation between the length of time spent studying the arts and improved SAT scores has been reported. Individuals who spent four or more years studying music and art scored an average of 59 points higher on verbal and 44 points higher on math portions of the SAT compared to students with no coursework or experience in the arts (MENC, Profiles of SAT and Achievement Test Takers, 1995).

It is evident that environmental enrichment can positively affect the biology and behavior of animals. More importantly, the concept of environmental enrichment first investigated in animals has influenced programs designed to enhance human behavior and development. The research suggests that humans are directly influenced by their environment and have benefited from environmental enrichment. Therefore, animal models are a valuable means to investigate the effects of environmental enrichment to inform the human condition.

**Body Weight**

Body weight is a quantitative measure that can be used to track developmental growth and to index health.  Excessive body weight, particularly among children, is a growing health concern in America.  The following section describes body weight and excessive body weight.  The measurement of body weight, the epidemiology, the consequences associated with excessive body weight, and body weight regulation are discussed.

*Description and Measurement*

Weight is the force of gravity on a mass, expressed as a number representing the mass' heaviness (Hewitt, 1997).  Accordingly, body weight is the force of gravity on an individual's body mass.  Body weight is the net result of daily energy intake and energy expenditure and typically increases as an organism grows.  When the daily intake of energy exceeds the amount of daily energy expended, body weight increases (Boon, Visser, & Daan, 1997; Warwick, Synowski, & Bell, 2002).  Excess energy is stored in the body as fat.  As energy intake exceeds the amount of energy expended, the amount of fat within the body increases and body weight increases.  The measurement of body fat is important because the presence of excess body fat increases risks for premature death or other negative health consequences (e.g., diabetes or cardiovascular disease).

Body weight varies greatly and depends on an individual's body composition.  Consequently, defining the "ideal," "normal," or "excessive" body for a given individual can be difficult.  Despite this fact, desirable weight

standards are typically determined in two ways.  The first method is to create a

weight table based on the actual heights and weights collected from a

representative or sample population.  These types of normative tables have been

developed by insurance companies (e.g., Metropolitan Life Insurance Company)

and by the National Center for Health Statistics (NCHS) (NRC, 1989; CDC, 2002)

for many years.  The NCHS identifies overweight individuals as persons whose

weights are at or above the 85th percentile of weight for height.  Those

individuals with weights represented by the top 5th percentile are considered

severely overweight or obese.  There are two major drawbacks to using this

normative sample approach.  First, the desirable weight standards change as the

weight distribution of the population changes.  Second, the underlying

assumption is that the "average" weight is a healthy or preferred weight and this

assumption may be inaccurate (Andres et al., 1985).   Health-care providers and

investigators studying body weight prefer the Body Mass Index (BMI) to

determine one's desirable body weight (NIH, 1995).  BMI is a mathematical

formula (a ratio of weight to height$^2$) that is highly correlated with body fat (Korner

& Aronne, 2003).  It is expressed as weight in kilograms divided by height in

meters squared (BMI = kg/m $^2$).   BMI is preferred because it correlates highly

with body fat, adjusts for height, and uses "cut-off" values associated with health

risks.

The definition of a "normal" or "healthy" BMI was recently changed in 2000.

Federal agencies have made a transition to define overweight as a BMI using the

recommendations in the current edition of Dietary Guidelines for Americans

(2000) (Kuczmarski & Flegal, 2000).  Consistent with internationally recommended BMI cut-offs, a BMI of < 25 kg/m$^2$ is considered normal, overweight is reflected by a BMI of 25 – 29.9 kg/m$^2$, and obesity is identified as a BMI of > 29.9 kg/m$^2$ (NCHS, Public Health Service, 2000; Korner & Aronne, 2003).  Prior to this change there was an inconsistent use of BMI cut-offs based on references and standards that affect the numbers of Americans classified as overweight and obese.  Kuczmarski and Flegal (2000) reported that when BMI cut-offs of ≥ 27.8 for men and ≥ 27.3 for women were applied to the National Health and Nutrition Examination Survey (NHANES) III data, the prevalence of overweight among adults aged ≥ 20 years was 33.3% for men and 36.4% for women.  In contrast, at a BMI ≥ 25.0, the prevalence was 59.4% for men and 50.7% for women.  By changing the overweight cut-offs, the estimated number of overweight adults increased from 61.7 million (BMI ≥ 27.8 and 27.3) to 97.1 million (BMI ≥ 25.0), representing a difference of 35.4 million overweight adults (Kuczmarski & Flegal, 2000).  Regardless of this change in categorization, excessive weight continues to increase at an alarming rate in the United States.

In addition to body weight, body fat can be inferred from a number of measures.  The most accurate means to measure body fat is through hydrodensitometry or hydrostatic weighing (HW); i.e., measuring body density via water displacement (Levenhagen et. al., 1999).  This method is cumbersome and time consuming, so easier, more efficient measures are often used.  Body fat can be estimated by skinfold measures, waist-to-hip circumference ratios, or techniques such as ultrasound, computer tomography (CT), magnetic resonance

imaging (MRI), bioelectrical impedance (BAI), or Body Mass Index (BMI) (NIH, 1995).  The most widely used measure to estimate body fat is BMI.

### *Excessive Body Weight, Description, Epidemiology, and Consequences*

Excessive body weight is described using the terms overweight and obesity.  Overweight refers to increased body weight in relation to height, when compared to some standard of acceptable or desirable weight (NIH, 1998).  Overweight may be the result of increases in body fat or it may be a result of an increase in lean muscle mass.  For example, professional athletes may be lean and muscular, with little body fat, yet they may weigh more than others of the same height.  Obesity is defined as an excessively high amount of body fat or adipose tissue in relation to lean body mass (NIH, 1998).

A high prevalence of overweight and obesity is of great public health concern.  The prevalence of obesity among adults has doubled in the past two decades (Flegal et al., 2002; Hedley et al., 2004).  Over 65 percent of Americans are estimated to be overweight or obese, defined as a BMI > 25 kg/m$^2$.  According to recent data (1999-2002), approximately 31% of adults $\geq$ 20 years of age (over 60 million people) had BMI $\geq$ 30 kg/m$^2$ compared to 23% in 1994 (Flegal et al., 2002).  The problem of excessive weight is not limited to the United States.  An estimated 1.7 billion people worldwide are overweight or obese (National Institutes of Health, 2004).

The number of children that are overweight or obese is reaching epidemic proportions in the United States.  The number of overweight children has doubled since 1980 (7% in 1980 to 16.5% in 1999–2002).  The number of overweight

adolescents has tripled in that same time period (5% in 1980 to 16% in 1999 –

2002) (Hedley et al., 2004). An additional 15 percent of children are considered

to be at risk for being overweight (Hedley et al., 2004). Excessive body weight in

children is particularly concerning because the risks of negative health

consequences increases the longer one has excessive body weight.

This increase in excessive body weight has severe negative health

consequences. Excessive body weight can result in elevated risks for premature

death and for many serious medical conditions, including: diabetes mellitus,

hypertension, dyslipidemia, cardiovascular disease, stroke, gall bladder disease,

respiratory dysfunction, gout, osteoarthritis, and certain kinds of cancers (Pi-

Sunyer, 2004). Two important factors associated with the risk of developing

several chronic diseases are total body fat and the distribution of the fat on the

abdomen and trunk or peripherally on the arms and legs (NIH, 1995). It is ironic

that obesity is increasing in the United States while more people are dieting than

ever before and spending $30 billion to $50 billion yearly on weight-reduction

products (including diet foods and soft drinks, artificial sweeteners, and diet

books) and services (e.g., fitness clubs and weight-loss programs) (Kassirer &

Angell, 1998). According to the recent figures, medical expenses for overweight

and obesity accounted for 9.1% of total U.S. medical expenditures in 1998 (Wolf,

1998; Finkelstein, Fiebelkorn, & Wang, 2004) and was estimated to cost $117

billion in the U.S. for the year 2000 (combination of direct health care costs plus

indirect costs, such as lost wages caused by illness) (U.S. Department of Health

and Human Services, 2001). It is estimated that Medicare and Medicaid

programs pay about $39 billion a year for treatment of medical conditions related to excessive weight gain (e.g., diabetes, cardiovascular disease, cancer) (Finkelstein, Fiebelkorn, & Wang, 2003). Clearly, excessive body weight is a critical problem in America.

### Body Weight Regulation

There are four interrelated factors that lead to body weight changes: metabolic activity (Bray & York, 1979; Westertep-Plantenga, 2004), genetics (Meyer & Stunkard, 1993), daily energy intake (Sclafani & Springer, 1976; Sclafani & Gorman, 1977; Warwick, Synowski, & Bell, 2002), and daily energy expenditure (Premack & Premack, 1963; Lattanzio & Eikelboom, 2003). Animal studies provide clear evidence that metabolic activity and genetics are important factors in excessive weight gain (Bray & York, 1979). The most clear-cut examples are animals with recessively inherited forms of obesity that had greater weight gain and fat deposits compared to their lean littermates, despite precise paired feeding (Cox & Powley, 1981). Further, biological defects (e.g., hypothalamic lesions) and the observation that obesity runs in families from twin and adoption studies also support the contention that metabolic activity and genetics are important factors to consider (Meyer & Stunkard, 1993). However, metabolism and genetics explain about half of the variance in body weight, whereas energy intake and intake account for the other half (Leamy et. al., 2005).

This doctoral research project focused on the behavioral effects of the environment on body weight in rats. As such, energy intake and physical activity

are most relevant to the project and are discussed in more detail. The following two parts of this section discuss the relationship of each variable (feeding and physical activity) to body weight.

***Energy Intake: Body Weight and Food Consumption***

Changes in food intake can produce changes in body weight (Sclafani & Springer, 1976; Forbes, 1987; Iossa, Lionetti, Mollica, Barletta, & Liverini, 1999). An increase in energy intake by increasing calories can lead to weight gain. An increase in calories can be accomplished in two ways: (1) by increasing the amount of food provided or portion size (Sclafani & Springer, 1976; Schachter & Rodin, 1974), or (2) by consuming foods of high caloric density (e.g., nuts, fast foods, potato chips, or cookies) (Iossa, Lionetti, Mollica, Barletta, & Liverini, 1999; Warwick, Synowski, & Bell, 2002). There is a linear relationship between the amount of increased food intake and change in body weight that allows weight gain to be predicted from the amount of excess caloric ingestion (Forbes, 1987).

In a classic study of starvation in humans, Keys and colleagues (1950) reported that reduced-calorie diets were associated with weight loss in normal, healthy volunteers. Additionally, calorie restriction in obese subjects reduced body weight and fat stores (Forbes, 1987). In animals, increasing total caloric intake causes them to increase body weight. There are several ways of increasing voluntary food intake in animals, including the provision of more palatable diets (Sclafani and Springer, 1976), the administration of calorically dense drinking solutions (Warwick, Synowski, & Bell, 2002; Sclafani 2004),

switching diets frequently (Collier, 1985), and increasing the fat content of the diet (Warwick, Synowski, & Bell, 2002). From both human and animal research, the evidence suggests that increases in body weight result from increased daily energy intake that exceeds daily energy expenditure through the amount and types of foods that are eaten.

The control of energy intake is dependent upon satiety and two related concepts, hunger and appetite. Satiety is the state of being full to or beyond capacity (Graaf et al., 2004). Satiety is controlled by hunger and appetite, responses to internal and external signals that are related to energy balance (Graaf et al., 2004). Hunger is the physiological need for food and appetite is the psychological motivation for food intake (Lasagna, 1988; Andersen, 1996). Hunger and appetite control rely on peripheral physiology and metabolic interactions in the brain. The regulation of body weight is a homeostatic system that involves a negative feedback loop between intake and compensated factors (De Castro & Plunkett, 2002). Examples of compensated factors include plasma levels of blood glucose, free fatty acids, body weight, Central Nervous System (CNS) insulin, and hypothalamic neuropeptide-Y (NPY). Circulating inhibitory signals, produced in proportion to fat mass, act on the brain to reduce or increase hunger, which in turn increases or decreases appetite (Connan & Stanley, 2003). In addition, inhibitory signals are generated in response to individual meals, which alter energy intake. Fulton et al. (2000) reported that leptin acts to attenuate the reward produced by self-stimulation in some areas of the brain but to enhance these effects in other areas. Leptin acts on the circuits influencing

appetite and energy available for expenditure and also acts on the motivation to eat (Connan & Stanley, 2003).  Therefore, appetite can control energy intake and can mediate weight changes.

Appetite control is accomplished at three levels: (1) patterns of eating behaviors, (2) intermediary physiological mechanisms, and (3) brain pathways (De Castro & Plunkett, 2002).  Patterns of eating behavior range on a continuum from early to late control.  Early control is accomplished via sensory information (e.g., an empty plate) followed by cognitive control (e.g., complex information processing regarding the current context).  Later control is accomplished through post-ingestive and post-absorbative behaviors (e.g., getting up from the table immediately after eating or sleeping after digestion).  Intermediary physiological mechanisms involve chemical signaling via several circulating peptides, including leptin, insulin, cholecystokinin (CCK), glucagon, neurotensin, and somatosatin (Connan & Stanley, 2003).  As a meal is consumed, the ingested food interacts with these intermediary mechanism receptors leading to release of peptides.

The last level of control is accomplished as the circulating peptides enter and activate central circuits within the brain.  The hypothalamus is the primary center for appetite control.  Within the hypothalamus, the arcuate nucleus (ARC), paraventricular nucleus (PVN), and the lateral hypothalamus are critical sites for the action of peripheral signals (Connan & Stanley, 2003).  Leptin, a signal of fat mass, and insulin, the hormone responsible for the breakdown of glucose, are the primary peripheral signals of energy homeostasis.  Both of these peptides are secreted in proportion to adipose tissue and act directly on the ARC and PVN of

the hypothalamus (Connan & Stanley, 2003). The ARC is implicated as essential in regulation of the energy balance because its destruction produces obesity (Olney, 1969). The PVN is implicated in weight control, its destruction results in weight gain, and electrical stimulation of the PVN decreases appetite (Weingarten et al., 1985). In addition, dopamine and the brain's reward system are implicated in food consumption. Specifically, dopamine release is decreased in association with increases in obtaining food and dopamine release is enhanced by food deprivation (Berridge & Robinson, 2003). ***Energy Expenditure: Body Weight and Activity***

Physical activity is important for weight control and affects distribution of body fat (Cortwright, Chandler, Lemon, & DiCarlo, 1997). By using energy and maintaining muscle mass, physical activity is a useful and effective adjunct to dietary management. Increased fat mass and obesity occur when energy intake exceeds total daily energy expenditure for a prolonged period (Leibel, Rosenbaum, & Hirsch 1995). One way total energy intake can exceed total daily expenditure is through reduced physical activity.

Physical activity is defined as bodily movement produced by the contraction of skeletal muscle that increases energy expenditure above the basal level. Physical activity can be categorized in various ways, including the type, intensity, and the purpose of the activity. Common activity types include occupational, household, leisure time, or transportation. An example of physical activity classified by intensity is high, medium, or low impact aerobics. To categorize the purpose of physical activity, leisure time activity can be subdivided

into categories such as competitive sports, recreational activities (e.g., hiking, cycling), and exercise training (Surgeon General's Report on Physical Activity and Health, 1996; USDHHS, 2001). An important distinction to make is between physical activity and exercise because these terms are often misused interchangeably. Physical activity differs from exercise in that exercise is planned, structured, and repetitive physical activity targeted to improve or maintain one or more components of physical fitness (Caspersen, Powell, & Christensen 1985).

The distinction between physical activity and exercise is relevant to the current investigations for two reasons. First, this project monitored physical activity as opposed to a forced exercise program (e.g., swimming or running wheel) in rats. Physical activity was used to examine what naturally happens to animal activity, food consumption, and body weight in response to various housing conditions. Previous research clearly demonstrates that animals forced to exercise lose weight (Premack & Premack, 1963; Boakes & Dwyer, 1997; Mueller, Herman, & Eikelboom, 1999; Lattanzio & Eikelboom, 2003) and the purpose of the project was to examine the animals' responses to environmental conditions. Second, exercise is one type of physical activity with the purpose of improving physical fitness and the animals' physical fitness was not being addressed in this study.

Increased physical activity in the absence of increased caloric intake leads to a decrease in body weight and a change in body composition in animals. The amount of caloric expenditure is proportional to these effects and this relationship

is more pronounced for male compared to female rats (Keesey & Powley, 1986).

Rats reared using two different photoperiod schedules, one with an 18-hour

sleep cycle and one with a shorter sleep cycle of 6 hours, showed differences in

energy expenditure. The animals exposed to the 18-hour sleep cycle (i.e., less

activity period) had lower daily energy expenditure, a higher rate of weight gain,

less lean body mass, and similar energy intakes compared to the animals in a 6-

hour sleep cycle (Boon, Visser, Daan, 1997). These findings support the

importance of energy expenditure as a critical variable to weight gain because

energy intake was similar in these studies. Animals given opportunities to

exercise (i.e., a running wheel) consistently weigh less compared with controls

not given opportunities to exercise (Premack & Premack, 1963; Boakes & Dwyer,

1997; Mueller, Herman, & Eikelboom, 1999; Lattanzio & Eikelboom, 2003).

These findings indicate that physical activity is an important variable in animal

body weight and that the environment the animal is in affects physical activity.

Together, the data on energy intake regarding food consumption, and the

data on energy expenditure regarding physical activity indicate that the

environment can exert a powerful effect on body weight. The data on food

consumption indicate that manipulation of the amount and type of available foods

can significantly affect body weight. The data regarding physical activity clearly

indicate that increasing physical activity so that energy expenditure exceeds

energy intake leads to weight loss. Varying components of the environment such

as available foods, lighting, and availability for physical activity are likely to be

important influences on body weight. For example, access to more physical

activity via a larger cage or the availability of different types of foods in these cages may be important environmental factors to consider in body weight changes.

**Environmental Enrichment and Body Weight**

Few studies have reported the effects of environmental enrichment on body weight. Environmental enrichment has been reported to decrease body weight in adult rats (Sclafani & Springer, 1976) and adolescent rats (Hellemens, Benge, & Olmstead, 2004; Tomchesson, 2004). However, enrichments effects on body weight have not been studied extensively. This section describes three experiments relevant to this research project, presents potential mechanisms of action of the effects of enrichment on body weight, and identifies several limitations of research reporting findings relevant to environmental enrichment effects on body weight.

Three studies provide the foundation for this research project: Sclafani and Springer (1976), Hellemens, Benge, and Olmstead (2004), and Tomchesson (2004). Sclafani and Springer (1976) studied weight gain in adult female, Sprague-Dawley rats given access to a variety of foods. Sixteen animals were placed in one of three conditions for 65 days: an isolation condition (where animals were individually housed in standard wire mesh cages, 15.5 cm X 25 cm X 17.5 cm), an active condition (where animals were housed in a small wire mesh cage attached to a running wheel), and an environmentally-enriched condition called a "complex environment." In the "complex environment" eight littermates were housed together in two large, three-level, wire mesh cages (45

cm X 47.5 cm X 67.5 cm) that included wooden, metal, and plastic objects in the upper two levels.

Regardless of their housing condition, animals provided with a "cafeteria diet" (cookies, potato chips, salami, marshmallows, etc.) gained more weight than did the rats that were provided standard chow.  The animals with access to the activity wheel (activity condition) gained less weight than did the rats in the isolated or complex environments (Sclafani & Springer, 1976).  Animals in the complex environment gained as much weight on the "cafeteria diet" as did the animals in the isolated condition, indicating that the type of foods available and that opportunity for physical activity are important factors in the animals' weight gain.  The authors suggested that a larger and more complex environment might limit the development of obesity in these adult rats but did not elaborate on the mechanisms (e.g., increasing activity or decreasing food intake).  Because the amount of activity is a critical variable in determining body weight, it is possible that providing larger cage sizes can allow greater availability for physical activity and may result in lower body weights by increasing energy expenditures.

Sclafani and Springer (1976) also reported that the activity group with access to a running wheel weighed significantly less compared to the other groups when all animals had access to bland foods.  Access to a running wheel also attenuated some of the weight gain in the animals fed the cafeteria diet. The findings from this study suggest that monitoring physical activity and controlling the types of available foods should be investigated together.  This study indicates that opportunities for activity help to control body weight in adult

female rats.  This experiment did not examine adolescent or male rats and did not measure home cage activity.

Hellemans, Benge, and Olmstead (2004) examined body weight in male, Long-Evans rats that were fed standard rat chow.  The adolescent rats (21 day old upon arrival) were placed into three different housing conditions: isolated, standard housing, and enriched (consisting of 12 litter mates and toys) for 12 weeks.  Isolation reared animals were housed singly in cages without physical stimuli (40 cm X 10 cm X 10 cm).  Rats reared in the standard condition were housed in pairs with no physical stimuli (40 cm X 10 cm X 20 cm).  Enriched animals were housed in a group of 12 rats in a large three-level cage (75 cm X 75cm X 180 cm).  Enriched animals had access to wheels and toys that were cleaned and replaced bi-weekly.  The rats reared in isolation gained more weight than did enriched animals.  Further, the animals in the enriched condition maintained lower rates of weight gain and overall lower weights for up to 20 weeks of age (Hellemans, Benge, & Olmstead, 2004).  The only measure of physical activity was open-field locomotion (in a chamber other than the housing environment), examined on four consecutive days, after approximately 15 weeks of environmental enrichment.  Animals reared in enriched environments had significantly less horizontal and vertical activity in the open field than did the animals reared in isolation.  It is not clear if the enriched animals weighed less because they ate less, engaged in more activity in the home cages than did the isolation animals, or had higher metabolisms.  The only physical activity data (locomotion) indicate that the enriched animals were less active than the isolated

animals, which would lead to the conclusion that enriched housed animals should weigh more, not less. The investigators did not report food consumption during rearing and did not measure **home cage** activity.

Tomchesson (2004) examined body weight, feeding, and open field activity in male, adolescent, Sprague-Dawley rats, housed in enriched and non-enriched environments. Rats were placed in an enriched or non-enriched environment for 24 days. In the non-enriched housing condition, animals were single-housed in standard polycarbonate rat cages with no objects in the cages (40 cm x 20 cm x 20 cm). In the enriched condition, animals were housed in groups of three, in larger polycarbonate cages (46 cm x 36 cm x 20 cm). A variety of objects (durable dog and cat toys including colored textured balls, rings, and bones) were placed in the cage to provide physical and tactile stimulation. All of the animals were allowed access to a standard rat chow during the experiment. The non-enriched reared rats gained more weight than did the enriched animals and this effect was significant after approximately two weeks of enrichment. By the end of the 4 weeks, compared with rats housed in non-enriched environments, rats housed in enriched environments weighed less (6% on average) (Tomchesson, 2004). Results of the Open Field trials suggested that the animals in the enriched housing moved less than animals in non-enriched housing, which was consistent with previous open field data. This experiment did not examine **home cage** activity, it did not examine female subjects, and it did not examine various types of foods.

Together, these three experiments suggest that: (1) environmental enrichment can decrease body weight gains when bland foods are available but may have limited effect when cafeteria diets are available, and (2) open field physical activity does not appear to play a role in the effects of environmental enrichment on body weight. From these experiments, it is not clear (1) what role total activity, including activity in the home cage, may plan in controlling body weight, or (2) whether there are sex differences in environmental enrichment effects on body weight.

### *Potential Mechanisms of Action*

There are several potential mechanisms for environmental enrichment to alter body weight. These mechanisms are directly related to the factors that can result in weight changes: genetics, metabolism, physical activity, and feeding. There is strong evidence for a genetic component to human obesity (e.g., the familial clustering and the high concordance of body composition in monozygotic twins). However, the role of genetic factors in human obesity is complex, determined by the interaction of several genes (polygenic) that work in combination with each other as well as with environmental factors (e.g., nutrients, physical activity) (Froguel & Boutin, 2001). There is no reported research examining environmental enrichment and genetics to suggest that environmental enrichment can modify body weight through genetic mechanisms in humans or animals. Genetic changes as a result of environmental manipulations typically have an extended time course that makes direct experimental examination unfeasible for this project. As such, detailed hypotheses regarding any potential

mechanisms of action for environmental enrichment to alter body weight through genetic variation is not reasonable to present at this time.

In contrast, there is evidence suggesting that environmental enrichment can affect metabolism.  Metabolism is a combination of physical and chemical processes in which the body cells synthesize protoplasm for growth and repair along with complex substances that are broken down into simpler compounds that produce energy essential for the functioning of body cells (Hirsch, Fairchild, & Rosenbaum, 1995).  Three factors determine metabolic rate: (1) basal metabolic rate (BMR), the normal rate at which the body uses energy without engaging in activity, (2) the total amount of calories the body uses daily, (3) the rate energy is burned during exercise and during the food digestion (Hirsch, Fairchild, & Rosenbaum, 1995).  Factors that affect metabolism include body size, age, growth, gender, amount of lean muscle tissue, amount of body fat, hormonal and nervous system controls, and amount of physical activity (Baghurst et al., 1992).

One way to increase physical activity and potentially increase metabolism may be by providing additional room for movement.  However, it is not clear that animals housed in environmental enrichment have higher levels of physical activity compared to animals housed in non-enriched environments.  In fact, the limited available data (i.e., in novel open field environments) suggest that animals housed in enrichment are less active than animals housed in non-enriched environments.  It is not clear if the animals take advantage of the opportunity for additional social and physical stimuli.  An increase in physical activity would

result in energy expenditure. When expenditure exceeds intake, weight is decreased. If exposure to environmental enrichment results in increased activity and energy expenditure, then it would be logical that weight would decrease. The relationship between total physical activity and environmental enrichment is not known because it has not been previously examined.

Another potential mechanism for enrichment's effect on body weight is by directly influencing energy intake or feeding. The regulation of body weight is a homeostatic system involving circulating inhibitory signals that act on the brain to reduce or increase hunger which, in turn, increase or decrease appetite (Connan & Stanley, 2003). These inhibitory signals are generated in response to individual meals and can depend on the macronutrient content of the energy consumed. For example, Saris (2003) reported that there is an overwhelming amount of evidence that the ratio of fat to carbohydrate in the diet is the primary factor in the macronutrient composition of the diet that causes over-consumption and that leads to weight gain. In animals, sweet and salty foods are consumed in greater quantities than standard rat chow (Sclafani & Springer, 1976). The availability of different foods within the environment is a potential factor in body weight changes. It has been reported that animals have a high preference for activity and prefer exercise to feeding (Premack & Premack, 1963; Boakes & Dwyer, 1997). Environmental enrichment provides physical and social interactions, which also may alter food consumption. Because environmental enrichment affects feeding, the extent to which environmental enrichment affects consumption of foods, particularly more desirable foods, is important to examine.

Changes within the brain are also a potential mechanism for the effects of environmental enrichment on energy intake. The increased size of the frontal cortex, a consequence of environmental enrichment, may improve processing of the peripheral signals to the ARC and PVN within the hypothalamus, the center for energy consumption in the brain. For example, chemical signals from peptides (such as leptin or insulin) may be more quickly processed because the increased neurocircuitry (i.e., increased dendritic branching and synaptic formations) provides more receptors for such signals. It is also possible that endogenous hormones or neurotransmitters that affect feeding and appetite are altered as a result of environmental enrichment. For example, dopamine release was reportedly increased as a result of food deprivation (Berridge & Robinson, 2003). In a recent investigation in our laboratory, there was an increase in dopamine D2 receptors found in the brains of animals housed in environmental enrichment compared to animals housed in non-enriched conditions (Elliott, Grunberg, & Thanos, personal communication, 2005). Animals in enriched environments may alter energy intake as a result of changes in dopamine availability or other neurotransmitters.

### Limitations in the Literature

The existing literature on rat models of environmental enrichment is extensive. However, few published experiments have examined environmental enrichment, body weight, food consumption, and physical activity. The few studies that have reported findings regarding these variables have three major limitations. First, no experiment examining the effects of enrichment on body

weight has directly compared male and female subjects. Males and females differ in their biology and react differently to environmental conditions (e.g., Cortright et al., 1997; Kolb et al., 1998; Curtis et al., 2004). For example, female rats housed in enriched environment have been reported to be more active than male rats housed in enriched environments in an open field arena (Elliot, 2004). There may be additional sex differences in the effects of environmental conditions on physical activity and body weight. The extent to which environmental factors may contribute to these behavioral differences has not been extensively examined, but may have important implications. Differences in feeding behaviors in response to environmental influences early in life may provide valuable information towards our understanding the current trend of childhood obesity in America.

Second, studies examining the effects of enrichment have not examined home cage activity of the animals. Physical activity is an important factor in whether or not an animal gains weight and the rate at which weight is gained. In studies of environmental enrichment, physical activity is typically measured only in response to being placed in a novel environment. The data from open-field locomotion suggest that environmentally enriched animals are less active and, therefore, should weigh more. This fact apparently opposes the effect of environmental enrichment to decrease body weight. One possible explanation is that enriched animals engage in more physical activity in their home cages. In contrast, it is possible that similar to their behavior in a novel environment, enriched animals habituate to their home cages, and that their activity in their

home cages decreases over time which would suggest a different cause for the decreased body weight (e.g., genetics, metabolism, feeding). Therefore, home cage activity in response to environmental enrichment needs to be systematically investigated.

A third limitation of environmental research on body weight is that the feeding behavior of adolescent animals reared in environmental enrichment has not been reported. The only investigation that reported the effects of environmental enrichment on food consumption used adult subjects (Sclafani & Springer, 1976). Sclafani and Springer (1976) reported that environmental enrichment did not offset overfeeding when adult rats were given access to a variety of foods. However, adolescent rats are particularly sensitive to the effects of environmental enrichment (Rosenzweig & Bennett, 1996; Kolb et. al., 1998) and feeding experiences of young rats has long lasting effects on food consumption as they age (Sefcikova & Mozes, 2002). Also, identifying whether different amounts of stimuli available in the environment interact with feeding behavior, activity, or gender may help to explain gender differences in body weight and may aid in the development of educational programs to treat overweight individuals. This doctoral research project was designed to address these limitations.

## SECTION II - RESEARCH EXPERIMENTS

### Overview

Three separate experiments were designed to examine the effects of environmental enrichment on feeding, activity, and body weight, in adolescent

male and female rats. Previous experiments reporting effects of enrichment on body weight had not: (1) examined home cage activity, (2) compared different enriched environments, and (3) compared the effects of different diets in male and female adolescent rats. This section begins with a description and rationale for each independent and dependent variable, a description of the experimental design and sample size, and an explanation of the data analytic strategy. Then, a description of the methods, results, and a discussion of the findings from each individual experiment are presented. This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Pub, 82-23, rev. 1985).

### Rationale for Independent Variables

#### *Environmental Enrichment (EE)*

The environment in which an animal lives has important and long-lasting biological and behavioral effects. In animal research, housing is a key factor that is often ignored because it is not a variable of interest to the investigator. In fact, a review of 339 peer-reviewed papers that reported animal investigations revealed that 43% of the papers did not mention cage size (Steyermark & Mueller, 2001). In addition to the size of the cage, the stimuli available in the cage also are important.

In their "cafeteria diet" study, Sclafani and Springer (1976) reported that environmental enrichment did not attenuate the rate of weight gain in adult female Sprague-Dawley rats when given access to a variety of foods. The

environmentally enriched condition, called a "complex environment," housed

eight littermates together in a large, three-level, wire mesh cage (45 cm X 47.5

cm X 67.5 cm) that included wooden, metal, and plastic objects in the upper two

levels.  An even larger environment reported in the literature is a series of three

large interconnected wire mesh cages (72 cm X 70 cm X 46 cm each) (Soffie,

Hahn, Tero, & Eclancher, 1999).   These paradigms provide opportunities for

social interaction and physical stimulation consistent with the concept of

environmental enrichment (Rosenzweig & Bennett, 1996; Woodcock &

Richardson, 2000).  Enriched environments differ from isolated environments in

the number of animals per cage and the number of objects per cage

(Rosenzweig & Bennett, 1996; Kolb, et al., 1998; Van Praag, et a., 1999; Varty et

al., 2000; Schrijver et al., 2002).

Three different forms of housing were used in the current research project:

(Non-Enriched "NON", Enriched "ENR", and Super-Enriched "SUP").  The NON

housing condition refers to housing animals singly in standard polycarbonate rat

cages (40 cm x 20 cm x 20 cm) with no additional objects.  The ENR housing

condition refers to housing groups of three animals together in larger

polycarbonate cages (46 cm x 36 cm x 20 cm) providing opportunities for social

interaction.  In addition, a variety of objects (durable toys including colored

textured balls, rings, and bones) were placed in the cage to provide physical and

tactile stimulation.  This particular enriched environment has been effective in

previous experiments in our laboratory assessing environmental effects on

performance and activity (Elliott, 2004; Tomchesson, 2004).  The SUP condition

refers to a larger group housing where 12 animals are housed in a three-level, galvanized steel cage (76 cm x 61 cm x 137 cm) providing more opportunities for physical and social interaction than the ENR condition.  A variety of objects (durable toys including colored textured balls, rings, and bones) were placed in the cage to provide physical and tactile stimulation.  This type of environment has been effective in experiments evaluating enrichment, feeding, body weight, and drug actions (Sclafani & Springer, 1976; Bowling, Rowlett, & Bardo, 1993; Hellemens et al., 2004).  The two types of environmental enrichment were included in this project to determine if the amount of available physical and social stimulation differentially affects body weight in rats.  It is possible that Sclafani and Springer (1976) did not provide enough space to make a difference on body weight with the cafeteria diet.  Alternatively, enriched environments may not offset the effects of cafeteria diets on body weight.

### Foods

The quality and quantity of available foods are important factors influencing energy intake, body adiposity, and body weight (Sclafani & Springer, 1976; Brown & Grunberg, 1995; Sclafani, 2001; Sefcikova & Mozes, 2002).  High fat diets promote greater caloric intake and weight gain than lower fat diets based on epidemiological studies, experimental manipulation of dietary fat content in human participants, and animal models of diet-induced overeating (Warwick, Synowski, Rice, & Smart, 2003).  By offering diets high in fat and/or sugar, overeating and obesity can be produced in rats (Sclafani & Springer, 1976).

Presumably, rats prefer diets high in fat and sugar because carbohydrates and fat taste better than bland or standard rat chow. Palatability and food taste have been reported as significant factors on total food consumption and weight gain in rats (Sclafani, 2004). However, other factors such as macronutrient content (e.g., protein, type of fat) (e.g., Levine, Kotz, & Gosnell 2003), age of specific nutritional experiences (e.g., Carughi, Carpenter, & Diamond, 1989; Sefcikova & Mozes, 2002), and housing (e.g., Brown & Grunberg, 1995; Lopek & Eikelboom, 2000; O'Conner & Eikelboom, 2000) also influence feeding behaviors.

Sclafani and Springer (1976) first reported that "sugar rich" and "tasty fat" foods marketed for human consumption produced pronounced weight gains in rats. This "cafeteria diet" allowed continuous access to foods that could be found in virtually any supermarket such as: sweetened condensed milk, chocolate chip and cream filled cookies, salami, cheese, bananas, marshmallows, milk chocolate, and peanut butter (Sclafani & Springer, 1976). In fact, adult female rats given constant access to these foods gained over three times as much weight as did standard chow fed controls. Sweet and fatty foods have been reported to elicit approach behavior and reinforce operant responding (Sclafani, 2004; Berridge & Robinson, 2003). Therefore, human supermarket foods that are high in fat and sugar have robust effects on two key variables in this project: food consumption and body weight.

*Environmental Enrichment and Foods*

Few studies have reported the effects of environmental enrichment on feeding. Sclafani and Springer (1976) reported that environmental enrichment did not attenuate the weight gain of adult female, Sprague-Dawley rats when given access to a variety of foods commonly eaten by humans. Sclafani and Springer called this diet the "cafeteria diet." Regardless of their housing condition (Complex [i.e., Enriched] or isolation [Non-Enriched]), animals fed the "cafeteria diet" ate more than did the standard chow fed rats and gained more weight. Although environmental enrichment did not attenuate the body weight gain when the animals had access to a variety of tasty foods, the animals in the complex environment that were given access to standard chow ate less than the animals in the isolated housing that had access to the standard chow. It is noteworthy that this experiment used adult rats, and the effects of environmental enrichment are typically investigated using adolescent animals because young animals are particularly sensitive to environmental enrichment (Rosenzweig & Bennett, 1996; Kolb et al., 1998). The present research included male and female adolescent rats to determine if environmental enrichment affects body weight of these subjects with access to various foods.

Additional evidence supports the effects of environmental enrichment to decrease food consumption. In an experiment examining the behavioral effects of environmental enrichment, 21-day-old male Sprague-Dawley rats were fed standard chow during a 4-week enrichment period. Animals in the environmental enrichment condition ate less than did animals in the non-enrichment condition

(Tomchesson, 2004). In this experiment, food consumption was used to monitor animal health and was not the primary focus of the research project.

Studies that did not directly examine environmental enrichment but manipulated components of an animal's environment have reported feeding changes. For example, paired housing (Lopak & Eikelboom, 2000), the introduction of a running wheel (Lattanzio & Eikelboom, 2003), alternating housing conditions (O'Conner & Eikelboom, 2000), and the use of smaller cages (Steyermark & Mueller, 2002) resulted in pronounced feeding suppression. It is clear from the literature that environmental manipulations can significantly affect feeding. While enriched environments appear to decrease the body weight of animals, it is unclear if the reduction in body weight is the result of decreased feeding or energy intake, or the result of an increase in activity. The available open-field data suggest that activity is decreased in enriched animals; however, no studies of environmental enrichment have examined feeding and home cage activity. The present experiments include measures of food consumption and activity in the home cages in addition to activity in open-field chambers.

### *Sex*

Males and females differ in their biology and they react differently to environmental conditions (e.g., Cortright, Chandler, Lemon, Lemon, & DiCarlo, 1997; Kolb, Forgie, & Gibb et al., 1998; Curtis et al., 2004). In humans, adult males have been reported to experience significant weight loss with exercise despite only mild energy deficits (Anderson et al., 1991). Conversely, females who regularly exercise typically maintain a stable weight despite more substantial

energy deficits (Mulligan & Butterfield, 1990). In animals, female rats have been reported to have a greater preference for salt (NaCl) and sweet solutions compared to male rats (Flynn, Schulkin, & Havens, 1993). Curtis and colleagues (2004) reported that female rats are less sensitive to higher concentrations of sodium and less sensitive to lower concentrations of sucrose solutions compared to male rats, suggesting gender differences in taste preferences. Gender differences also have been reported in activity. Female rats subjected to exercise training (swimming, treadmill) gain weight at approximately the same rate, as do sedentary controls (Applegate, 1982). Conversely, males subjected to regular exercise typically lose weight (Oscai & Holloszy, 1969). Additionally, male rats allowed daily access to a running wheel reduced body mass and body fat compared to female rats allowed the same access to a running wheel.

Sex differences in feeding behavior have been reported in the animal research literature with some mixed results. Grunberg, Popp, and Winders (1988) investigated the effects of nicotine on body weight, consumption of potato chips, cookies, and standard chow in rats. Prior to the experimental manipulation of nicotine, adult female Sprague-Dawley rats ate less bland food than did adult male, Sprague-Dawley rats (4.45 g vs. 10.66 g, respectively) (Grunberg, Popp, & Winders, 1988). Further, there were no significant differences in the types of foods consumed by males and females. Males and females ate similar amounts of cookies (11.42 g vs. 10.44 g) and potato chips (2.32 g vs. 2.14 g) (Grunberg, Popp, & Winders, 1988). Additionally, adult female rats fed high-fat and high-carbohydrate diets ate more high-fat foods and became fatter than adult male

rats (Sclafani & Gorman, 1977).  Given the recent trend of excessive body weight currently being reported in adolescents, it is important to examine consumption of various types of foods in adolescents.  It also is important to determine whether environmental enrichment alters these behaviors.

### *Enrichment Effects and Sex Differences*

The effects of enriched environments have been investigated primarily using male subjects.  A few studies have compared the performance of males and females raised in enriched and non-enriched environments.  Male and female rats raised in enriched environments exhibit enhanced performance on a reference memory task compared to rats raised in non-enriched environments (Einon, 1980).  Male and female rats raised in enriched environments also made fewer memory tasks errors than did rats raised in non-enriched environments (Seymore, Dou, & Juraska, 1996).  Examining spatial memory performance using only females, Daniel, Roberts, and Dohanich (1999) have obtained similar results.

The effects of social environment on locomotion, feeding, acoustic startle, and pre-pulse inhibition (PPI) in male and female Sprague-Dawley rats suggest that females are more sensitive than are males to the behavior-altering effects of group housing (i.e., social enrichment) (Brown & Grunberg, 1995; Faraday, Rahman, Scheufele, & Grunberg, 1998; Faraday, Scheufele, Rahman, & Grunberg, 1999).  Results of a recent study conducted in our laboratory suggest that males and females differ in their sensitivity to physical and social aspects of the environment.  Elliott (2004) reported that males and females responded

differently to the effects of social and physical enrichment on a variety of tasks of information processing (i.e., acoustic startle response and pre pulse inhibition), simple working memory (i.e., passive avoidance), and complex spatial learning and memory (i.e., Morris water maze). Social enrichment enhanced performance of males and females on a simple information-processing task (i.e., locomotor habituation). Physical enrichment enhanced performance on the same measure for males, but not for females. Social enrichment improved performance on a complex spatial memory (i.e., Morris water maze) for males, but not for females. Physical enrichment improved sensory gating (i.e., % PPI) for females, but not for males. The present research project, therefore, included male and female subjects.

## Rationale for Dependent Variables

### *Body weight (BW)*

Body weight (BW) gain is the net result of daily energy intake and energy expenditure. BW increases when the amount of daily energy intake exceeds the amount of daily energy expended (Boon, Visser, Daan, 1997; Warwick, Synowsky, & Bell, 2002). In animals, BW is a basic biologic measure that can be used to index health, monitor aging, and evaluate growth (e.g., Sclafani and Springer, 1976; Brown & Grunberg, 1995; Faraday & Grunberg, 2000). Within the rodent literature, experimental subjects are typically described using two criteria: (1) the age of the animal reported as the number of days since birth (e.g., 21 days old), and (2) the animal's body weight in grams (e.g., 300 grams).

BW is a widely used measure in studies of: social isolation (Hall, Humby, Wilkinson, & Robbins, 1997), social crowding (Brown & Grunberg, 1995), physical and social stimulation (Sclafani & Springer, 1976; O'Conner & Eikelboom, 2002), immobilization stress (Faraday, 2002), and various pharmacologic agents (Grunberg et al., 1985; Bowling et al., 1994). Of particular interest to this project, studies examining feeding behaviors that manipulate food availability or food/nutrient choice routinely use BW as a dependent variable (e.g., Sclafani & Springer, 1976; Hall, Humby, Wilkinson, & Robbins, 1996; Boakes & Dwyer, 1997; Lopek & Eikelboom 2000; Sefcikova & Mozes, 2002; Lattanzio & Eikelboom, 2003).

In addition, BW can be used to monitor animal health. For example, "crowding" animals can provide stress (Bowen & Grunberg, 1995) or competition for food that prevents animals from receiving necessary nutrients. A continuous lack of nutrients can result in poor health and retarded growth. Insufficient nutrition that occurred in young rats during their first three weeks of life resulted in decreased body weight and decreased body growth when compared to normally fed controls (Bartness et al., 1987). Conversely, excess body weight can indicate poor health. Excessive weight gain can be induced in rats by offering them foods that are high in fat and sugar through foods usually used for human consumption such as cream filled cookies, marshmallows, milk chocolate, potato chips, and peanut butter (Sclafani & Springer, 1976; Grunberg, Bowen, Maycock, & Nespor, 1985; Sclafani 2004).

Excessive weight gain increases risk for diseases such as coronary heart disease, diabetes, hypertension, respiratory problems, and some types of cancer (Flegal et al., 2002). BW is a simple, non-invasive measure that is sensitive to environmental manipulations. Additionally, BW is a face-valid measure used with humans and animals that can be measured repeatedly in the same animals and analyzed using the mixed experimental design (Brown & Grunberg, 1995; O'Conner & Eikelboom, 1999; Faraday, 2002).

### Body mass index (BMI)

BMI is a measure expressing the relationship (or ratio) of weight-to-height commonly used to classify a human's weight as healthy or unhealthy. BMI is calculated by using the following formulas:

$$\text{BMI} = \underset{\text{(in Kilograms)}}{\text{Body Weight}} \div \underset{\text{(in meters)}}{\text{Height}^2}$$

OR

$$\text{BMI} = 705 \text{ X } (\underset{\text{(in pounds)}}{\text{Body Weight}} \div \underset{\text{(in inches)}}{\text{Height}^2})$$

The "height" of the rat was measured from the tip of the nose to the end of the rump defined as the beginning of the tail. BMI is more highly correlated with body fat than any other indicator of height and weight (NRC, 1989). Further, BMI is a reliable and valid measure of adiposity in adults (Garrow & Webster 1985) and children (Zimmermann, Gubeli, Puntener, & Molinari, 2004).

In rodents, few studies have examined BMI. Increases in BMI are correlated with increasing levels of leptin in rats (Maffei et al., 1995; Engelbregt et al., 2001). The hormone leptin is the central mediator in a negative feedback loop regulating energy homeostasis (Engelbregt et al., 2001). Leptin

administration leads to reduced food intake, increased energy expenditure, and weight loss **(**Cohen & Friedman, 2004).  Additionally, weight loss as a result of food restriction has been associated with a decrease in plasma leptin (Maffei et al., 1995).  Engelbregt et al. (2001) reported that BMI was correlated with body mass and percentage of fat in 24 day old female but not male Wistar rats.  BMI is easily obtainable and has been correlated with the percentage of body fat in rats (Maffei et al., 1995**)**.  This research project used the formula presented by Engelbregt and colleagues (2001) to calculate BMI:

$$BMI = Body\ Weight \div Height^2$$
$$\text{(in grams)} \qquad \text{(in cm)}$$

### *Lee Index (LI)*

Similar to BMI, the LI is a ratio of weight-to-height used to report adiposity in rats.  It is a non-invasive and simple measure that is used in rodent research on obesity and feeding.  Using the same measurements taken to calculate the animal's BMI, LI is the cubic root of body weight in grams divided by the naso-anal length in millimeters times $10^4$.  The naso-anal length of the rat is measured from the tip of the nose to the end of the rump, defined as the beginning of the tail.  LI is highly correlated with body fat and has proven to be a reliable and valid measure of adiposity in rats (Lee, 1929; Bernardis, Luboshitsky, Bellinger, & McEwen, 1982; Emsberger, P., Koletsky, P.J., Baskin, J. S., & Collins, L.A., 1996).

Several studies have reported LI as a reliable measure of adiposity. Straub (2003) reported that adult Wistar rats that were fed a normal diet and

administered drugs to reduce weight over a four-week period had significantly

lower LI scores compared the animals that did not receive the weight-reducing

drugs.  Bernardis and colleagues (1982) reported that Lee Index scores for

obese male Sprague-Dawley rats (that received bilateral electrolytic lesions in

the ventromedial hypothalamic nuclei [VMNL rats] to induce obesity) were

greater than were LI scores of control rats (that received a sham-operation).  In a

population of normal and obese mice, Rogers and Webb (1980) reported that the

Lee Index correlated significantly with body fat and several other measures used

to indicate obesity in mice (body density, body water, and proportional weight of

the gonadal fat pad).  This research project used the formula presented by Lee

(1929) to calculate the LI scores:

$$LI = [(g\ body\ wt)^{1/3} / (mm\ body\ length)] \times 10^4$$

### Food consumption (FC)

Similar to body weight (BW), food consumption (FC) can affect health.

Extensive experimental data indicate that feeding behaviors can be profoundly

affected by environmental manipulation.  For example, stressors such as the tail

pinch (Faraday, 2002) and repeated cold stress (Schultz et al., 1999) increase

food intake, whereas restraint (Zylan & Brown, 1996; Faraday, 2002) and

crowding (Brown & Grunberg, 1995) reportedly decrease feeding.

The role of housing in feeding behaviors is not as clear.  Perez et al.

(1997) reported that individually housed rats ate more than did rats housed in

groups.  In contrast, moving rats from individual to paired housing has been

reported to decrease food intake for several days and feeding was not altered

when the rats were placed from their paired housing back into individual housing

(O'Conner & Eikelboom, 2000).   Placing rats in isolation at the weaning period

increased food consumption (Fiala, Snow, & Greenough, 1977), but placing rats

in isolation after weaning has been reported to decrease feeding (Yamada et al.,

2000).  The feeding behavior of rats appears to be particularly sensitive to early

environmental manipulation.  When mothers were insufficiently nourished,

consequent malnourishment of the rat pups led to changes in feeding when the

pups were adults.  Specifically, males that were underfed during lactation feeding

ate more standard rat chow during adulthood compared to the well-fed controls

(Smart & Dobbing, 1977).  Undernourished female pups were smaller and

weighed less in adulthood, but did not significantly over-eat on a diet of standard

rat chow (Sefcikova & Mozes, 2002).

Although the directionality of environmentally-driven feeding changes may

not be clear, the fact that feeding changes are sensitive to environmental

manipulations is important.  If a clearly established feeding pattern is altered by a

given manipulation, then it is clear that the manipulation has produced a change

in behavior.  This change in feeding can have health consequences, regardless

of whether the resultant change is an increase or decrease in feeding.  These

reported differential responses in feeding parallel human feeding.  For example,

in the DSM IV T/R (2001), one criterion for depression is a change in feeding and

body weight -- Directionality is not an important distinction.  Further, stress and

anxiety can result in increased or decreased appetite  (Zylan & Brown, 1996;

Brown & Grunberg, 1995; Faraday, 2002).  Again, the difference from baseline

consumption is the key feature of the criterion (DSM IV T/R, 2001).

FC is a simple, non-invasive measure that is sensitive to environmental

manipulations.  Additionally, similar to BW, FC is a face-valid measure used with

humans and animals (Brown & Grunberg, 1995; O'Conner & Eikelboom, 1999;

Faraday, 2002) that can be measured repeatedly in the same animals and

analyzed using the mixed experimental design of the present experiments.

### *Physical Activity (PA)*

### *Home cage activity (HCA)*

Home cage activity refers to the animal's behavior in its primary living

quarters.  Previous researchers have reported a seemingly paradoxical finding

that rats reared in environmental enrichment compared to rats reared in non-

enriched environments appear to have less activity (at least in novel

environments) (e.g., Van Wass & Soffie, 1996; Paulus, Bakshi, & Geyer, 1998)

but weigh less (O'Conner & Eikelboom, 2000; Hellemans, Benge, & Olmstead,

2004).  It is noteworthy to mention that these "enriched" rats were healthy

animals because rats that were ill would be expected to be less active, eat less,

and, therefore, weigh less.  Consequently, it was important to examine if animals

reared in the enriched and non-enriched environments differ in the amount of

activity within their home cages.  An extensive literature search of the

PsychInfo® and National Library of Medicine (PubMed®) databases revealed no

studies that have examined home cage activity in enriched environments.

Therefore, two observational methods were developed and used to assess home cage activity. The two methods are described in the methods of Experiment I.

***Open field (OF)***

Open-field locomotion describes an animal's behavior when it is placed in a non-home cage arena. Behaviors relevant to environmental enrichment include activity in the horizontal plane, vertical plane, and center time. Level of activity and frequency of rearing behaviors have been used to index an animal's habituation to a novel environment (Varty et al., 2000; Bowling et al., 1993; Van Waas & Soffie, 1996). Habituation, a simple form of learning, refers to the progressive reduction in responding to a novel stimulus when the stimulus is repeatedly presented to a subject (Varty et al., 2000). A decrease in overall activity or rearing behaviors suggests habituation or efficient processing of novel information. Conversely, an absence of behavioral changes over time reflects decreased information processing of relevant environmental stimuli (Varty et al., 2000; Bowling et al., 1993; Van Waas & Soffie, 1996).

Relevant to this project, animals housed in enriched environments reduce locomotor activity, reduce exploration over time, and have more restricted movements in open field testing when compared to non-enriched animals (van Wass & Soffie, 1996; Paulus, Bakshi, & Geyer, 1998; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001; Varty et al., 2000). These findings suggest that rats housed in enrichment assimilate information from their environment and adapt more effectively to novel environments than do rats raised in non-enriched environments. This decrease in activity, however, is not consistent with the

research reported that environmental enrichment decreases the rate of weight gain in rats (O'Conner & Eikelboom, 2000; Hellemans, Benge, & Olmstead, 2004; Tomchesson, 2004). Based on energy expenditure alone, if activity decreased, then the weight of the animals should increase. Home cage activity has not been carefully examined and compared to open-field activity within the context of environmental enrichment. This research project examined the effects of environmental enrichment on activity in both the novel open-field environment and home cage.

### Experimental Design and Determination of Sample Size

All three experiments were conducted using a 7-week, repeated-measures, mixed (i.e., within-subject and between-subjects) factorial, A-B-A design with 12 subjects per cell. Experiment I and Experiment II examined the effects of environmental enrichment on feeding, weight gain, and activity in male and female adolescent Sprague-Dawley rats, respectively. Experiment III examined male and female subjects together to attempt to replicate the findings from the first two experiments and to allow a direct comparison of sex. Animals were housed in one of three types of housing, representing three different levels of environmental enrichment: Non-Enriched (NON), Enriched (ENR), and Super-Enriched (SUP). All subjects were given access to different types of foods during the different phases of the experiment (A = Standard Food, B = Standard Food plus Salty plus Sweet Junk Foods). During Phase A, all subjects had access to standard rat chow. During Phase B, all subjects had access to standard rat chow a sweet food (Oreo™ cookies), and a salty food (Lay's™ plain potato chips).

Three behavioral measures were used: open-field locomotion, home cage activity, and food consumption.  In addition body weight was measured and a body mass index score and a Lee Index score were calculated for each animal at the end of each study.

The sample sizes were determined based on previous reports using similar dependent measures and responses to environmental enrichment. Similar studies in the research literature, which reported statistically significant effects, had cell sizes of 7 – 12 animals for enrichment (e.g., Van Praag et al., 1999; Elliott & Grunberg, 2005; Tomchesson, 2004).   Mering Kaliste-Korhonen and Nevalainen (2000) determined that 5 - 10 animals were needed to find statistically significant effects for enrichment on various biological measures including body weight and fat/adipose tissue.  The exact experimental design and sample size of Experiment III was determined after completing Experiment II.

A sample size of 12 animals was selected to optimize statistical power across a range of dependent measures that vary in effect size in response to environmental enrichment and weight and was determined based on studies in the existing literature.  Sample sizes were confirmed using the procedures of Keppel (1991) and Cohen (1988).  Estimates of effect size in the population were determined to provide 0.80 power by calculating an estimated omega squared ($\varpi^2$) according to the formula:

$$\varpi^2_A = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_{S/A}}$$

where $\varpi^2_A$ refers to the estimated population treatment effects and  $\sigma^2_{S/A}$ refers to the estimated population error variance (Keppel et al., 1992, p. 180).  The omega

squared statistic provides a measure of effect size that is relatively independent of sample size and is expressed as a proportion of the total variability ($\sigma^2_A$ + $\sigma^2_{S/A}$) that is associated with the treatment or manipulation ($\sigma^2_A$).

## Data Analytic Strategy

The goal of this project was to examine if environmental conditions affect activity, feeding, body weight, body fat, and the extent to which any effects may vary as a function of sex. For all animals, food consumption (FC), BMI, and LI were analyzed with separate analyses of variance (ANOVAs) with Enrichment (housing condition) as the between-subjects factors. Body weight (BW) was analyzed with repeated-measures analyses of co-variance (ANCOVA) with Enrichment as the between-subjects factor and Time as the within-subject factor. Baseline body weight was used as a covariate to compensate for the variance of body weights within each experimental group throughout the various experiments. Home cage activity (HCA) was analyzed using the Kruskal-Wallis chi-square because HCA data were interval data with more than two independent experimental groups. For the open-field (OF) measures, horizontal and vertical activity was first analyzed with a multivariate analysis of variance (MANOVA) to determine if there was an overall difference between groups across the two related OF measures. If the MANOVA results revealed significant differences on the OF measures, then separate univariate analyses of variance (ANOVA) for each OF variable were performed. During the analyses, Tukey HSD tests or, when a covariate was used, a pairwise post-hoc comparison using a Bonferroni correction for multiple comparisons was used to compare housing conditions if

there was a significant main effect for housing type. The computer-based statistical analyses automatically adjusted the alpha levels following the Bonferroni correction procedures to maintain a true alpha of 0.05. For Experiment III, a repeated-measures analysis of variance (ANOVA) was used with Sex and Enrichment (housing condition) as the between-subjects factors and Time as the within-subject factor.

All tests were two-tailed with alpha = 0.05. Several strategies were employed to minimize the probability of Type 1 error. First, the experiment was designed with enough subjects to provide adequate power (i.e., 0.80). When the sample size is large enough to support adequate power, the likelihood of Type I errors is minimized. Second, global analyses incorporating all factors (Housing Condition and Sex) were used to guide internal analyses. Sub-group analyses followed only if overall analyses revealed significant main effects or interactions. This strict Fisherian strategy is consistent with recommendations of Keppel (1991) and Cohen (1988), and reduces the number of statistical tests performed. Finally, the error term (the within-subject variance that constitutes the denominator of the F ratio) specific to the comparison being made was used rather than the error term for all subjects. This technique controls Type I error because as the denominator degrees of freedom decrease, the F value necessary to achieve significance for a given comparison increases. The following section presents the experimental results for each dependant variable and provides an individual discussion of the results for each of the three experiments in this project. *Note:* The following abbreviations are used for the

three housing conditions: "NON" = Non-Enriched; "ENR" = Enriched; "SUP" = Super-Enriched housing.

## Experiment I

Experiment I examined the effects of environmental enrichment on activity, feeding, and body weight in male, adolescent, Sprague-Dawley rats. The experiment was a 7-week investigation that used a repeated-measures, mixed, A-B-A design. Subjects were housed in one of three environmental conditions (NON, ENR, and SUP) for the duration of the experiment and all subjects were given access to different types of foods during the different phases of the experiment (A = Standard Food, B = Standard Food plus Salty plus Sweet Junk Foods). The goals of Experiment I were to examine the effects of environmental enrichment on: (1) body weight when different foods are available, (2) food consumption (variety of foods), (3) physical activity (open field arena and home cage).

### *Hypotheses: Experiment I*

### *Hypothesis 1 – EE, BW, BMI, and LI*

A) Environmental enrichment will decrease body weight gains, Lee index, and Body Mass index such that: Non-Enriched > Enriched > Super-Enriched.

*Rationale.* Environmental enrichment has been reported to decrease the rate of weight gain in rats (O'Conner & Eikelboom, 2000; Hellemans, Benge, Olmstead, 2004; Tomchesson, 2004).

*Hypothesis 2 – EE and FC*

A) Environmental enrichment will decrease the amount of bland foods consumed compared to animals reared in Non-Enrichment such that:  Non-Enriched > Enriched > Super-Enriched.

*Rationale.*  Rats housed in environmental enrichment eat less bland food (Sclafani & Springer, 1976; Tomchesson, 2004).

B) Animals raised in enriched and non-enriched housing conditions will eat similar amounts of cafeteria foods such that:  Non-Enriched = Enriched = Super-Enriched.

*Rationale.*  Rats housed in environmental enrichment did not eat less cafeteria food than singly housed controls (Sclafani & Springer, 1976).

*Hypothesis 3 – EE and PA*

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Non-Enriched > Enriched > Super-Enriched.

*Rationale.*  Enriched animals exhibit reduced locomotor activity and reduced exploration over time compared to non-enriched animals (Van Wass & Soffie, 1996; Paulus, Bakshi, & Geyer, 1998; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001; Varty et al., 2000).

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Non-Enriched < Enriched < Super-Enriched.

***Rationale.*** Animals exposed to longer activity periods have higher daily energy expenditure (Boon, Visser, Daan, 1997) and animals that are allowed access to exercise (i.e., a running wheel) choose exercise over feeding (Premack & Premack, 1963; Boakes & Dwyer, 1997; Mueller, Herman, & Eikelboom, 1999; Lattanzio & Eikelboom, 2003). Therefore, the animals in the enriched housing conditions that have more opportunity for physical activity will be more active.

## *Methods: Experiment I*

### *Subjects*

Subjects were 36 adolescent male (21 days old at the beginning of the experiment) Sprague-Dawley rats (Charles River Laboratories). Adolescent animals were used to maximize the developmental impact of environmental environment and because of the investigator's interest in child and adolescent development. Sprague-Dawley rats were used because they are the most commonly used strain of outbred albino rats. Twelve subjects were randomly assigned to each of the three experimental treatment conditions upon arrival.

### *Housing*

All animals were housed on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at approximately $23^0$ C and approximately 50% relative humidity on a 12-hour reversed light/dark cycle (lights off at 0530 hours). A reversed light cycle was maintained to ensure that all behavioral measures were made during the animals' normal activity period. Animals were assigned to one of three housing conditions (Non-Enriched "NON", Enriched

"ENR", and Super-Enriched "SUP").  In the NON condition, animals were single-housed in standard polycarbonate rat cages (40 cm x 20 cm x 20 cm) with no additional objects.  In the ENR condition, animals were housed in groups of three in larger polycarbonate cages (46 cm x 36 cm x 20 cm), which provided opportunities for social interaction.  In addition, a variety of objects (durable toys including colored textured balls, rings, and bones) were placed in the cage to provide physical and tactile stimulation.  Objects were removed every other day (or sooner if damaged) and replaced with new objects.  In the SUP condition, 12 animals were housed together in a three-level, galvanized steel cage (76 cm x 61 cm x 137 cm), which provided more opportunities for physical activity and social interaction than the ENR condition.  A variety of objects (durable toys including colored textured balls, rings, and bones) also were placed in the cage to provide physical and tactile stimulation.  The objects used, changing schedule, and cage dimensions were based on methods described in previous studies (Gardner et al., 1975; Sclafani & Springer, 1976; Bowling et al., 1993; Varty et al., 2000; Elliott, 2004; Tomchesson, 2004).  See Figures 1-3 in the Appendix for pictures of housing conditions.

***Procedures***

On experimental Day 1 (upon arrival to the laboratory), animals were randomly assigned to one of the three housing conditions for the experiment's duration.  The experiment was conducted in three experimental phases using an A-B-A design.  Each experimental phase corresponds to a change in the animal's

available food.  All animals, regardless of housing condition, had access to the same foods.

The first two experimental phases each lasted two weeks, and the third phase lasted three weeks.  The entire experiment lasted a total of 50 days. Table 1 in Section IV presents the experimental Timeline.  During Phases A1 and A2 (Days 1-14 and 30-50, respectively), all animals had access to standard rat chow.  During Phase B (Days 15-29), all subjects were allowed to eat standard rat chow and, in addition, had access to a sweet food (Oreo™ cookies) and a salty food (Lay's™ plain potato chips).  The letters A and B identify the experimental phase, the numbers 1 and 2 differentiate between the two A phases, and the number of days spent in the phase designate the experimental days during each of the phases (e.g., A1-1 is the first day of the first phase where animals received standard chow).  All animals were acclimated to the open-field (OF) activity chambers on Day 2 to minimize contamination of responses by any stressful effects of exposure to a novel situation (Faraday & Grunberg, 2000). Acclimation procedures do not affect later measurement of OF habituation.  All behavioral measures were conducted between 0800 and 1300 hours (the dark/active cycle).  This period of time was used to maximize behavioral performance and physical activity because rats are nocturnal and more active during the dark cycle.

### Dependent Variables

**Body Weight (BW).**   BW was measured on Day 1 and then three times during each two-week experimental phase (the 5th, 10th, and 14th day) and two

additional times for Phase A2 (the 18$^{th}$ and 21$^{st}$ day). Animals were removed

from their cages and gently placed on an electronic scale. To ensure accurate

weight measurements (i.e., reduce measurement error), the electronic scale

automatically obtains multiple weight readings and provides an average of these

readings. These procedures provided 12 body weight measures (four during

Phase A1, three during Phase B, and five during Phase A2).

*Body Mass Index (BMI).* BMI was measured on the final day of the

experiment. At the end of the experiment, body weight (BW measurement 12)

and naso-anal length in millimeters were measured and documented. BMI was

calculated using these values.

*Lee Index (LI).* LI was measured on the final day of the experiment. At

the end of the experiment, body weight (BW measurement 12) and naso-anal

length in millimeters were measured and documented. LI was calculated using

these values.

*Food Consumption (FC).* Food weights were measured every other day

providing a total of 21 Food Consumption measurements. Animals had

continuous access to food. Standard, bland, food pellets were placed on the top

of each cage for NON and ENR conditions and in four separate food containers

placed inside the cage for the SUPER condition. The salty and sweet foods were

provided in 4 inch metal food cups with lids and placed in the cages in alternating

positions to avoid the development of place preferences or aversions. Food

Consumption was determined by subtracting new food weights from previous

food weights (e.g., subtracting Day A1-3 food weights from Day A1-1).

Whenever food was added to the containers, the new weight was recorded and this new weight was used in the next calculation.  Six Food Consumption values were calculated for the animals during each two-week experimental phase (nine values calculated for Phase A2).  To appropriately describe food consumption for this investigation, both the weight of food consumed (in grams) and caloric intake during each experimental phase were examined.  The number of grams consumed was examined to provide a simple measure to compare the amount of food eaten. The number of calories consumed was examined to allow a direct comparison of energy intake (caloric density of foods can vary depending on weight and macronutrient content of the foods).  Each variable was examined separately during each phase because the available foods differed during each phase (standard chow for Phases A1 & A2 and standard chow, salty, and sweet during Phase B).

*Physical Activity (PA).*  Two different methods of behavioral observation were developed to examine activity in home cages. The first method (Individual Animal Activity) was designed to examine animal activity for each individual animal.  The second method (Group Activity) was designed to examine an aggregate level of activity for each experimental housing group.  Each method is described in detail.  (See figures 4 and 4a for HCA Data Sheets.)

Home Cage Activity (HCA) Method 1 – Individual Animal Activity.
Individual animal activity was observed three times during the first two-week experimental phase of Experiment I (the $2^{nd}$, $7^{th}$, and $14^{th}$ day).  Two independent observers quietly observed each animal in home cages.  The room was dimly lit

with red light. Each observer watched each animal for 3 minutes and recorded horizontal and vertical locomotion on a 7-point Likert format scale. The order of rats observed was balanced across housing conditions and the time of observation was varied during the dark cycle for each day of observation. The level of effort expended during each activity period also was judged and rated on a 7-point Likert format scale. In addition, the type of physical activity that each animal was engaged in was recorded (e.g., with a physical object, combined social and physical interaction, or alone).

This technique was initially tested using a small group of five adult animals from a previous experiment in our laboratory. The two independent observers were trained until an inter-rater reliability of at least 80% was obtained for at least five consecutively observed animals. Individual activity was to be examined at least three times during each experimental A-B-A phase (the beginning, middle, and end). However, it became immediately evident that this measure was not providing meaningful data. In less than 10 minutes after the observers entered the housing room, all of the animals appeared to decrease their activity levels. Within 30 minutes after entering the room, typically all of the animals would stop moving and lay down. In addition, it was nearly impossible for the observers to keep track of all of the animals' individual activity in the larger cage using this Individual Animal Activity technique.

The observers also tried leaving the room for over an hour and then returning to observe activity again. When the observers returned, the animals were active again and the observers again attempted to record activity. This

time, the observers quickly recorded a rating of "overall" activity for the group of animals in each experimental housing condition.  The rationale was for the observers to obtain a "snap shot" of physical activity as soon as they entered the room, prior to attempting to get individual animal activity ratings.  As before, the animals' physical activity ceased within 30 minutes after the observers entered the housing room.  However, preliminary analyses examining the "group" activity measures suggested that group activity provided meaningful data with which to compare the three experimental housing groups.  An additional attempt to measure individual animal activity was made and a group activity measure was recorded prior to individual observations.  Once again, the group activity recordings provided meaningful data to compare the experimental groups. Therefore, a "group activity" scale was developed and used for the remainder of Experiment I.

*Home Cage Activity (HCA) Method 2 – Group Activity.*  Group activity was observed a minimum of five times during experimental phases B and A2.  The measure was developed during the first phase, so no group activity measures are available for Phase A1.  Two independent observers quietly observed animals in home cages and provided a global rating for each experimental housing group.  The room was dimly lit with red light.  Each observer watched each animal for one minute and recorded the number of animals engaged in physical activity, and average horizontal, vertical, and center cage locomotion activity on a 7-point Likert format scale.  An average level of effort expended during each activity period also was judged and rated on a 7-point Likert format

scale. In addition, the type of physical activity that each animal was engaged in was recorded (e.g., with a physical object, combined social and physical interaction, or alone). The order of rats observed was balanced across housing conditions and the time of observation was varied for each day of observation. The observations all were made during the 12-hour dark cycle, but the observation time within this cycle was varied for each observation day.

Group activity was observed at random times to sample activity. The activity was monitored during the animals' dark cycle/active period to maximize the potential to observe the animals engaging in physical activity. In addition, the observers alternated the order in which the groups were observed to reduce any biases and confounds associated with the amount of time the observers were in the room (as seen when the observers were recording individual activity). These procedures were used for the remainder of Experiment I.

Open-Field (OF). OF activity was measured at the beginning and end of each experimental phase (Days A1-4, A1-13, B1-3, B1-13, A2-3, and A2-20). Open-Field activity was measured using an Omnitech Electronics Digiscan infrared photocell system (Test box model RXYZCM [16 TAO]; Omnitech Electronics, Columbus, OH). Animals were placed singly in a 40 x 40 x 30 cm clear Plexiglas arena and a Plexiglas lid with multiple 3.5 cm diameter holes was placed on top of the arena. The lid ensures that subjects have adequate ventilation but cannot escape during data collection. A photocell array measures horizontal activity using 16 pairs of infrared photocells located every 2.5 cm from side-to-side and 16 pairs of infrared photocells located front-to-back in a plane 2

cm above the arena floor.  A second side-to-side array of 16 pairs of additional photocells located 10.5 cm above the arena floor measures vertical activity.  Data are transmitted to a computer via an Omnitech Model DCM-I-BBU analyzer. (See Figure 6 in Section IV for a picture of an OF arena.)

Once subjects are placed in the test arenas, the experimenter turns off the lights and leaves the room.  The apparatus monitors animal activity continuously for a total testing period of 1 hour. The interfaced software generates 21 sub-variables, including horizontal and vertical activity (measures of activity in the horizontal plane and exploratory activity, respectively).  Horizontal activity and vertical activity were analyzed as measures of general activity, habituation, and exploration.

### Results: Experiment I

### Body Weight (BW)

Body weight was measured 12 times during the experiment (four times during Phase A1, three times during Phase B, and five times during the Phase A2).  An overall ANCOVA revealed a main effect for Time ($F$ (10, 320) = 8.185, $p$ < 0.05), main effect for housing ($F$ (2, 32) = 3.474, $p$ < 0.05), and a Time X Housing interaction ($F$ (10, 320) = 4.601, $p$ < 0.05).  Post hoc comparisons revealed that animals housed in Non-Enriched cages weighed more than animals in Enriched cages that weighed more than animals in the Super-Enriched cages (NON>ENR>SUP).  Post-hoc pairwise comparisons using a Bonferroni adjustment for multiple comparisons revealed that animals housed in Super-Enriched cages weighed significantly less than animals housed in Non-Enriched

cages, but the differences in the weights of animals housed in Non-Enriched vs. Enriched housing as well as Enriched vs. Super-Enriched were not significant (NON$\geq$ENR>SUP).  There were no significant differences in body length between the three groups (See Table 22).  (See Table 2 and Figures 7a- 7c for Body Weight results.)

### Body Mass Index (BMI)

BMI was computed based on body weight and length at the end of the experiment corresponding BW measurement 12.  BMI values were significantly related to housing conditions ($F$ (2, 33) = 6.682, $p$ < 0.05).  Tukey HSD post hoc analyses revealed that animals in the NON housing condition had significantly higher average BMI than the animals raised in the ENR condition, which had higher average BMI than, but was not significantly different from, the SUP condition (NON>ENR$\geq$SUP).  (See Table 3 and Figure 8.)

### Lee Index (LI)

LI was computed based on body weight and length at the end of the experiment using measurement 12.  Animals housed in enrichment had significantly lower LI values compared to animals housed in no enrichment ($F$ (2, 33) = 4.919, $p$ < 0.05).  Tukey HSD post hoc analyses revealed that animals in the NON housing condition had significantly higher average LI values than the animals raised in the ENR condition, which had higher average LI values that were not significantly different from the SUP condition (NON>ENR$\geq$SUP).  (See Table 4 and Figure 9.)

*Food Consumption (FC)*

FC was measured 25 times during Experiment I (seven measurements for Phase A1 & B and 11 measurements for Phase A2). Average grams and calories consumed daily were examined separately for each experimental phase because the available foods differed for each phase (i.e., during Phases A1 & A2, only standard chow was available and during Phase B, standard chow, salty, and sweet foods were available). The results for grams and calories are presented separately for each phase of the experiment.

*Phase A1 – Standard Chow Only Period: GRAMS.* There was a significant main effect for housing ($F$ (2, 33) = 3.835, $p$ < 0.05). A Tukey HSD comparison revealed that the difference between animals housed in Super-Enriched conditions and animals housed in both Non-Enriched and Enriched conditions approached significance (0.056 and 0.057, respectively), but there was no difference between animals raised in Non-Enriched and Enriched conditions (NON$\geq$ENR>SUP). (See Table 5a and Figure 10a.)

*Phase A1 – Standard Chow Only Period: CALORIES.* There was a significant main effect for housing ($F$ (2, 33) = 3.835, $p$ < 0.05). A Tukey HSD comparison revealed that the difference between animals housed in Super-Enriched conditions and animals housed in both Non-Enriched and Enriched conditions approached significance (0.056 and 0.057, respectively), but there was no difference between animals raised in Non-Enriched and Enriched conditions (NON$\geq$ENR>SUP). (See Table 5b and Figure 10b.)

***Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: GRAMS.*** There were no significant differences in the average amount of food consumed daily during Phase B.  (See Table 5c and Figure 10a.)

***Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: CALORIES.***  Animals in the enriched conditions ate less than animals housed in the non-enriched conditions and the difference between housing conditions approached significance during Phase B (0.068).  (See Table 5d and Figure 10b.)

Specific Food Types examined separately:

***Phase B – Standard Chow: GRAMS.*** There was a significant main effect for housing ($F$ (2, 33) = 3.767, $p$ < 0.05) (NON<ENR<SUP).  A Tukey HSD comparison revealed animals housed in Super-Enriched conditions ate significantly more food than animals in the Non-Enriched condition but that there were no significant differences in the amount of standard chow eaten between animals raised in Super-Enriched housing and Enriched or between animals raised in the Non-Enriched and Enriched conditions (**NON**<ENR<**SUP**).  (Note: Bold type indicates groups that were significantly different.)  (See Table 5e and Figure 10c.)

***Phase B – Standard Chow: CALORIES.***  There was a significant main effect for housing ($F$ (2, 33) = 3.767, $p$ < 0.05).  A Tukey HSD comparison revealed that animals housed in Super-Enriched ate significantly more standard chow calories than animals in the Non-Enriched condition but that there were no significant differences in the amount of standard chow eaten between animals

raised in the Non-Enriched and Enriched conditions (**NON**<ENR<**SUP**). (Note: Bold type indicates groups that were significantly different.)  (See Table 5f and Figure 10d.)

*Phase B – Cookies: GRAMS*. There were no significant differences in the average amount of cookies consumed.  (See Table 5g and Figure 10c.)

*Phase B – Cookies: CALORIES*. There were no significant differences in the cookie calories consumed (See Table 5h and Figure 10d.).

*Phase B – Potato Chip: GRAMS.* There were no significant differences in the average amount of potato chips consumed.  (See Table 5i and Figure 10c.)

*Phase B – Potato Chip: CALORIES*.  There were no significant differences in the caloric intake of potato chips.  (See Table 5j and Figure 10d.)

*Phase A2 – Standard Chow Only Period: GRAMS*. There were no significant differences in the amount of standard chow consumed during Phase A2.  (See Table 5k and Figure 10a.)

*Phase A2 – Standard Chow Only Period: CALORIES*.  There were no significant differences in the average number of calories consumed during Phase A2.  (See Table 5l and Figure 10b.)

*Physical Activity (PA)*

*Home Cage Activity (HCA).*  HCA was measured 17 times during Experiment I (six times during Phase B and 11 times during Phase A2).  The average number of animals moving, amount of activity, and level of activity were observed during each measurement.  Each type of HCA is presented for each experimental phase.

*Phase B – Standard Chow with Sweet and Salty Foods Period*.  The Kruskal-Wallis Chi-Square revealed significant differences for number of animals moving, amount of activity, and level of activity.  Kruskal-Wallis mean rank orders indicated that the enrichment conditions had the most animals moving: NON<ENR<SUP; the most amount of home cage activity: NON < ENR < SUP; and higher levels of activity: NON<ENR<SUP.  (See Table 6a and Figures 11a – 11c.)

*Phase A2 – Standard Chow Only Period.*  The Kruskal-Wallis Chi-Square was significant for number of animals moving, amount of activity, and level of activity.  Kruskal-Wallis mean rank orders indicated that the enrichment conditions had the most animals moving: NON<SUP<ENR; the most amount of home cage activity: NON<ENR<SUP; and higher levels of activity: NON<ENR<SUP.  (See Table 6b. and Figures 11a. - 11c.)

***Open Field (OF).***  A total of six OF trials examining horizontal, vertical, and center-time activity were conducted during Experiment 1 (two per experimental phase A1, B, and A2).  Overall MANOVAs revealed significant differences between housing conditions in total horizontal activity and vertical activity.  Therefore, only the differences in horizontal and vertical activity were then examined using repeated-measures ANOVAs for each individual Open-Field trial.  The results of the between-groups and the within-session analyses are presented separately for each Open-Field trial.  Tables 7a – 7l present the details of the statistical analyses for the Open-Field trials.   Figures 12a - 12d

present graphical depictions of the within-session horizontal and vertical activity for Open-Field trials.

*Phase A1 – Standard Chow Only Period, Open-Field Trial 1 Between Groups.* Horizontal activity (F $(2,33)$ = 7.716, p < 0.05) and vertical activity (F $(2,33)$ = 3.362, p < 0.05) were significantly different between housing conditions. Post Hoc pairwise comparisons revealed significantly lower horizontal activity in the SUP compared to ENR and NON (NON>ENR≥SUP). Although between-subjects effects indicated a significant difference for vertical activity (F $(2,33)$ = 3.362, p < 0.05), the post hoc pairwise analyses revealed no significant differences between any of the three housing conditions. (See Tables 7b & 7c and Figures 12a – 12d.)

*Phase A1 – Standard Chow Only Period, Open-Field Trial 1 Within Groups Within Session.* Repeated-measures ANOVAs revealed significant main effects for time (within session) on horizontal activity ($\underline{F}$ $(11, 363)$ = 30.459, $\underline{p}$ < 0.05) and vertical activity ($\underline{F}$ $(11, 363)$ = 14.704, $\underline{p}$ < 0.05) indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity ($\underline{F}$ $(22, 363)$ = 3.826, $\underline{p}$ < 0.05) and vertical activity ($\underline{F}$ $(22, 363)$ = 2.504, $\underline{p}$ < 0.05) indicated that the decrease in activity over time was different between the three housing conditions. Post Hoc pairwise comparisons revealed that the SUP housing condition activity was significantly lower than ENR, and NON (NON≥ENR>SUP). (See Tables 7b & 7c and Figures 12a – 12d.)

*Phase A1 – Standard Chow Only Period, Open-Field Trial 2 Between Groups.* Horizontal activity ($F_{(2,33)}$ = 20.947, $p < 0.05$) and vertical activity ($F_{(2,33)}$ = 14.120, $p < 0.05$) were significantly different between housing conditions. Post Hoc pairwise comparisons revealed that the SUP housing condition was significantly different from the ENR and NON housing conditions (NON≥ENR>SUP). (See Tables 7d & 7e and Figures 12a – 12d.)

*Phase A1 – Standard Chow Only Period, Open-Field Trial 2 Within Groups Within Session.* Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F_{(11, 363)}$ = 47.499, $p < 0.05$) and vertical activity ($F_{(11, 363)}$ = 39.802, $p < 0.05$) indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity ($F_{(22, 363)}$ = 6.348, $p < 0.05$) and vertical activity ($F_{(22, 363)}$ = 5.035, $p < 0.05$) indicated that the decrease in activity over time was different between the three housing conditions. Post Hoc pairwise comparisons revealed that the amount of SUP animal's activity was significantly different from the ENR and the NON housed animals (NON≥ENR>SUP). (See Tables 7d & 7e and Figures 12a – 12d.)

*Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 3 Between Groups.* Horizontal activity ($F_{(2,33)}$ = 13.394, $p < 0.05$) and vertical ($F_{(2,33)}$ = 8.966, $p < 0.05$) were significantly affected by housing condition. Post Hoc pairwise comparisons revealed that the SUP condition was significantly different from the ENR and NON housing conditions (NON≥ENR>SUP). (See Tables 7f & 7g and Figures 12a – 12d.)

*Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 3 Within Groups Within Session.* Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F$ (11, 363) = 47.521, $p$ < 0.05) and vertical activity ($F$ (11, 363) = 40.548, $p$ < 0.05), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity ($F$ (22, 363) = 4.47, $p$ < 0.05) and vertical activity ($F$ (22, 363) = 2. 408, $p$ < 0.05), indicated that the decrease in activity over time was different between the three housing conditions. Post Hoc pairwise comparisons revealed that the amount of activity was significantly lower in the SUP compared to the ENR and the NON housed animals (NON≤ENR<SUP). (See Tables 7f & 7g and Figures 12a – 12d.)

*Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 4 Between Groups.* Horizontal activity ($F$ (2,33) = 9.604, $p$ < 0.05) and vertical activity ($F$ (2,33) = 6.186, $p$ < 0.05) were significantly different between groups. Post Hoc pairwise comparisons revealed significantly lower activity for SUP and ENR compared to NON (NON>ENR≥SUP). (See Tables 7h & 7i and Figures 12a – 12d.)

*Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 4 Within Groups Within Session.* Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F$ (11, 363) = 85.290, $p$ < 0.05) and vertical activity ($F$ (11, 363) = 53.655, $p$ < 0.05), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity ($F$ (22, 363) = 4.475, $p$ < 0.05)

and vertical activity ($F_{(22, 363)}$ = 2.693, $p < 0.05$) indicated that the decrease in activity over time was different between the three housing conditions.  Post Hoc pairwise comparisons revealed that the amount of activity was lowest in the SUP followed by the ENR, and the NON housed animals had the greatest activity (NON<ENR<SUP).  (See Tables 7h & 7i and Figures 12a – 12d.)

*Phase A2 – Standard Chow Only Period, Open-Field Trial 5 Between*

*Groups.*  Horizontal activity ($F_{(2,33)}$ = 24.785, $p < 0.05$) and vertical activity ($F_{(2,33)}$ = 13.373, $p < 0.05$) were significantly different.   Post Hoc pairwise comparisons revealed significant differences between all three groups for horizontal activity (NON>ENR>SUP) and no differences between the SUP and ENR groups for vertical activity (NON>ENR=SUP).  (See Tables 7j & 7k and Figures 12a – 12d.)

*Phase A2 – Standard Chow Only Period, Open-Field Trial 5 Within*

*Groups.*  Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F_{(11, 363)}$ = 80.477, $p < 0.05$) and vertical activity ($F_{(11, 363)}$ = 60.300, $p < 0.05$) indicating that animal activity decreased over time in all conditions.  In addition, a significant time by housing interaction for horizontal activity ($F_{(22, 363)}$ = 3.057, $p < 0.05$) and vertical activity ($F_{(22, 363)}$ = 2.763, $p < 0.05$) indicated that the decrease in activity over time was different between the three housing conditions.  Post Hoc pairwise analyses revealed that the amount of activity was lowest in the SUP followed by the ENR, and the NON housed animals had the most activity (NON<ENR<SUP).  (See Tables 7j & 7k and Figures 12a – 12d.)

*Phase A2 – Standard Chow Only Period, Open-Field Trial 6 Between*

*Groups.*   Horizontal activity ($F_{(2,33)}$ = 16.079, $p$ < 0.05) and vertical activity ($F_{(2,33)}$ = 11.735, $p$ < 0.05) were significantly different between groups.  Post Hoc pairwise comparisons revealed lower horizontal and vertical activity for SUP and ENR compared to NON (NON<ENR$\leq$SUP).  (See Tables 7l & 7m and Figures 12a – 12d.)

*Phase A2 – Standard Chow Only Period, Open-Field Trial 6 Within*

*Groups Within Session.*  Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F_{(11, 363)}$ = 72.093, $p$ < 0.05) and vertical activity ($F_{(11, 363)}$ = 41.351, $p$ < 0.05), indicating that animal activity decreased over time in all conditions.  In addition, a significant time by housing interaction for horizontal activity ($F_{(22, 363)}$ = 3.840, $p$ < 0.05) and vertical activity ($F_{(22, 363)}$ = 2.230, $p$ < 0.05) indicated that the decrease in activity over time was different for the three housing conditions.  Post Hoc pairwise comparisons revealed that the amount of activity was lowest for the SUP followed by the ENR, and the NON housed animals had most activity (NON<ENR<SUP).   (See Tables 7l & 7m and Figures 12a – 12d.)

**Open-Field Results Summary.**  Animals in the enriched housing conditions were less active during each of the six OF trials compared to the non-enriched condition.  The difference between the three housing conditions indicated that the animals housed in the NON condition moved the most, followed by ENR, and the SUP housed animals moved the least.  These differences in activity in the housing conditions are consistent with other studies

(Bowling, Rowlett, & Bardo, 1993; Van Waas & Soffie, 1996; Varty et al., 2000; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001). (See Figures 12a – 12d for graphical representations of averaged activity.)

## *Discussion: Experiment I*

Experiment I examined the effects of environmental enrichment on body weight, feeding, and activity in male, adolescent, Sprague-Dawley rats. This experiment was designed to examine the effects of environmental enrichment on: (1) body weights when the different foods are available, (2) food consumption, using bland foods and other, more preferred foods, and (3) physical activity, including activity in an open field arena and within the home cage.

Environmental enrichment was associated with lower body weights over the seven-week experiment. This finding is consistent with previous research (e.g., Sclafani & Springer, 1976; Tomchesson, 2004). Additionally, animals housed in enriched conditions had significantly lower BMI and LI scores at the end of the experiment compared to the animals in the non-enriched housing. These lower indices suggest that animals housed in enriched environments had lower amounts of body fat (Maffie et al., 1995).

Enrichment appeared to decrease the amount of food consumed for standard chow early in the experiment. This decreased food intake would be consistent with lower body weights and body fat. The decreased feeding became less marked as different types of foods (sweet & salty) were made available, as time went by, and as the subjects aged. Clearly, the amount of standard chow consumed decreased when the cookies and chips were offered,

but there were no significant differences between housing conditions in the amount of standard chow or potato chips eaten during Phase B.

Physical activity was measured in novel open field environments and in home cages. Open Field activity (horizontal and vertical activity) was lower for the animals housed in enrichment and was the lowest for the animals housed in the SUP condition. The effect for enrichment to decrease OF activity is consistent with previous reports (e.g., O'Conner & Eikelboom, 2000; Varty et al., 2000; Elliott 2004; Tomchesson, 2004). This effect for enrichment to decrease activity in the OF is not consistent with a decrease in body weight or body fat. It is consistent with effects of enrichment on learning (decreases in OF activity may indicate improved habitation a simple form of learning) (Varty et al., 2000). Conversely, animals in enriched environments exhibited more activity in the home cages compared to animals in non-enriched housing. The increased amount and intensity (i.e., level) of activity in the home cage is consistent with decreases in body weight. Further, home cage activity was consistent with the differential decreases in body weight seen in the different housing conditions (i.e., BW = NON>ENR$\geq$SUP and HCA = NON<ENR$\leq$SUP). The findings from Experiment I examining three different types of environmental enrichment with varying amounts of physical and social activity suggest an increased effect of increased levels of environmental enrichment. Specifically, more opportunities for physical and social stimulation resulted in higher amounts and levels of activity in the home cage and lower body weights. It is clear from Experiment I that environmental enrichment decreased body weight, had a small effect on

feeding, and led to increased activity in the home cage for male rats. However, it was not clear if there were differences in the amounts of the different types of foods eaten when the animals had a choice because the difference between housing conditions in the amount of cookies eaten did not reach significance.

## Experiment II

Experiment II examined the effects of environmental enrichment on body weight, feeding, and activity (open-field activity and home cage activity) in female, adolescent, Sprague-Dawley rats. Experiment II was conducted using the procedures of Experiment I except that the subjects were adolescent female rats. The "group activity" technique to evaluate home cage activity that was developed during Experiment I was used for this experiment. A detailed description of "group activity" is provided in the methods section of Experiment I.

Identical to Experiment I, the goals of Experiment II were to determine the effects of environmental enrichment on: (1) body weight when different types of foods (sweet and salty) are available, (2) food consumption (bland foods and other foods), and (3) physical activity (open field arena and home cage).

### *Hypotheses: Experiment II*

### *Hypothesis 1 – EE, BW, BMI, and LI*

Environmental enrichment will decrease body weight gains and body mass index such that: Non-Enriched > Enriched > Super-Enriched.

*Rationale.* Environmental enrichment has been reported to decrease the rate of weight gain in rats (O'Conner & Eikelboom, 2000; Hellemans, Benge, Olmstead, 2004; Tomchesson, 2004).

*Hypothesis 2 – EE and FC*

A) Environmental enrichment will decrease the amount of bland foods consumed compared to animals reared in Non-Enrichment such that:  Non-Enriched > Enriched > Super-Enriched.

*Rationale.*  Rats housed in environmental enrichment eat less bland food (Sclafani & Springer, 1976; Tomchesson, 2004).

B) Animals raised in enriched and non-enriched housing conditions will eat similar amounts of cafeteria foods such that:  Non-Enriched = Enriched = Super-Enriched.

*Rationale.*  Rats housed in environmental enrichment did not eat less cafeteria food than singly housed controls (Sclafani & Springer, 1976).

*Hypothesis 3 – EE and PA*

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Non-Enriched > Enriched > Super-Enriched.

*Rationale.*  Enriched animals reduced locomotor activity and reduced exploration over time compared to non-enriched animals (van Wass & Soffie, 1996; Paulus, Bakshi, & Geyer, 1998; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001; Varty et al., 2000).

 B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Non-Enriched < Enriched < Super-Enriched.

***Rationale.*** Animals exposed to longer activity periods have higher daily energy expenditure (Boon, Visser, Daan, 1997) and animals that are allowed access to exercise (i.e., a running wheel) choose exercise over feeding (Premack & Premack, 1963; Boakes & Dwyer, 1997; Mueller, Herman, & Eikelboom, 1999; Lattanzio & Eikelboom, 2003). Therefore, animals in the enriched housing conditions, that have more opportunity for physical activity, will be more active.

### *Methods: Experiment II*

The methods and design of Experiment I were used in Experiment II with few modifications. Specifically, female subjects were used and home cage activity was measured using the group activity measure only.

### *Subjects*

Subjects were 36, adolescent (21 days old on arrival), female Sprague-Dawley rats, resulting in 12 subjects per cell.

### *Housing*

Housing conditions were identical to Experiment I. All animals were housed on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001 or "Junk" Foods) and water. Animals were assigned to one of three housing conditions (NON, ENR, or SUP) as described in Experiment I.

### *Procedures*

The procedures in Experiment II were the same as in Experiment I with one exception. Only the group activity measure was used to measure home cage activity. This change was made because during Experiment I, measuring

group activity provided a more accurate and logistically feasible measurement of physical activity than did the individual activity measure. A detailed description of the "group activity" measure and rationale is provided in the methods of Experiment I. (See Table 8 in Section IV.)

### Dependent Variables

The dependent variables were identical to Experiment I.

**Body Weight (BW).** BW was measured on Day 1 and then three times during each two-week experimental phase (the $5^{th}$, $10^{th}$, and $14^{th}$ day) and two additional times for Phase A2 (the $18^{th}$ and $21^{st}$ day).

**Body Mass Index (BMI).** BMI was measured on the final day of the experiment.

**Lee Index (LI).** LI was measured on the final day of the experiment.

**Food Consumption (FC).** Food weights were measured every other day providing a total of 21 Food Consumption measurements.

**Physical Activity (PA).** _Home Cage Activity – Group Activity_. Group activity was observed a minimum of five times during experimental phase B and A2 only.

Open-Field (OF). OF activity was measured at the beginning and end of each experimental phase (Days A1-4, A1-13, B1-3, B1-13, A2-3, and A2-20).

### Results: Experiment II

### Body Weight (BW)

Body weight was measured 12 times during the experiment (four times during Phase A1, three times during Phase B, and five times during the Phase

A2).  An overall ANCOVA revealed a main effect for Time ($F$ (10, 320) = 12.554, $p$ < 0.05) and a main effect for housing ($F$ (2, 32) = 7.827, $p$ < 0.05), and a Time X Housing interaction ($F$ (10, 320) = 3.082, $p$ < 0.05).  Post-hoc pairwise comparisons using a Bonferroni adjustment for multiple comparisons revealed that the body weight of animals in the Super-Enriched condition was less than the Enriched and the Non-Enriched conditions.  Additionally, animals housed in Super-Enriched cages weighed significantly less than animals housed in Non-Enriched cages, but the differences in the weights of animals housed in Non-Enriched and Enriched housing were not significant (NON≥ENR>SUP).  There were no significant differences in body length between the three groups (See Table 22).  (See Table 9 and Figures 13a -13c for Body Weight results.)

### Body Mass Index (BMI)

A significant main effect for housing ($F$ (2, 33) = 6.836, $p$ < 0.05) and Tukey HSD post hoc analyses revealed that animals in the SUP housing condition had significantly lower average BMIs compared to animals raised in the ENR condition and NON housing.  Animals in the ENR and NON conditions were not significantly different (NON≥ENR>SUP).  (See Tables 10 and Figure 14.)

### Lee Index (LI)

A significant main effect for housing ($F$ (2, 33) = 4.342, $p$ < 0.05) and Tukey HSD post hoc analyses revealed that animals in the SUP housing condition had significantly lower average LI's than the animals raised in the ENR condition and the NON conditions (NON≥ENR>SUP).  (See Table 11 and Figure 15.)

***Food Consumption (FC)***

Food consumption (FC) was measured 25 times during Experiment I (seven measurements for Phase A1 & B and 11 measurements for Phase A2). Average grams and calories consumed daily were examined separately for each experimental phase because the available foods differed for each phase (i.e., only standard chow was available during Phases A1 & A2 and standard chow, salty, and sweet foods were available during Phase B).  The results for grams and calories are presented separately for each phase of the experiment.

***Phase A1 – Standard Chow Only Period: GRAMS.***  There was a significant main effect for housing ($\underline{F}$ (2, 33) = 3.835, $\underline{p}$ < 0.05).  Tukey HSD post-hoc comparisons revealed that animals housed in Super-Enriched conditions and in Enriched conditions ate significantly fewer calories than did animals in the Non-Enriched housing but there was no difference between animals housed in Super-Enriched and Enriched conditions (NON>SUP$\geq$ENR).  (See Table 12a and Figure 16a.)

***Phase A1 – Standard Chow Only Period: CALORIES.***  There was a significant main effect for housing ($\underline{F}$ (2, 33) = 3.767, $\underline{p}$ < 0.05).  Tukey HSD post-hoc comparisons revealed that animals housed in Super-Enriched conditions and in Enriched conditions ate significantly fewer calories than did animals in the Non-Enriched housing, but there was no difference between animals housed in Super-Enriched and Enriched conditions (NON>SUP$\geq$ENR).  (See Table 12b and Figure 16b & 16c.)

***Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: GRAMS.*** There were no significant differences. (See Table 12c and Figure 16c.)

***Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: CALORIES.*** There were no significant differences. (See Table 12d and Figure 16d.)

***Phase B – Standard Chow: GRAMS.*** There were no significant differences. (See Table 12e and Figure 16c.)

***Phase B – Standard Chow: CALORIES.*** There were no significant differences. (See Table 12f and Figure 16d.)

***Phase B – Cookies: GRAMS***. There was a significant difference in the average amount of cookies consumed ($F_{(2, 33)}$ = 13.899, $p < 0.05$). Super-Enriched housed animals ate significantly less cookies than Enriched housed animals, that ate significantly less than animals housed in the Non-Enriched conditioned (NON>ENR>SUP). (See Table 12g and Figure 16c.)

***Phase B – Cookies: CALORIES***. There was a significant difference in the average number of cookie calories consumed ($F_{(2, 33)}$ = 13.899, $p < 0.05$). Super-Enriched housed animals ate significantly less cookie calories than Enriched housed animals, that ate significantly less cookie calories than animals housed in the Non-Enriched conditioned (NON>ENR>SUP). (See Table 12h and Figure 16d.)

***Phase B – Potato Chip: GRAMS***. There were no significant differences. (See Table 12i and Figure 16c.)

***Phase B – Potato Chip: CALORIES***.  There were no significant differences.  (See Table 12j and Figure 16d.)

***Phase A2 – Standard Chow Only Period: GRAMS***. There were no significant differences.  (See Table 12k and Figure 16a.)

***Phase A2 – Standard Chow Only Period: CALORIES***.  There were no significant differences.  (See Table 12l and Figure 16b.)

***Physical Activity (PA)***

***Home Cage Activity (HCA).***  HCA was measured 17 times during Experiment II (five times during Phase A1, five times during Phase B, and seven times during Phase A2).  The average number of animals moving, amount of activity, and level of activity were observed during each measurement.  Each type of HCA is presented for each experimental phase.

*Phase A1 – Standard Chow Only Period.*  The Kruskal-Wallis Chi-Square was not significant for number of animals moving, but was significant for amount of activity, and level of activity.  Kruskal-Wallis mean rank orders indicated that all enrichment conditions were similar with regard to the mean number of animals moving NON=ENR=SUP, but that animals in the SUP condition had the most activity: NON<ENR<SUP, and had higher levels of activity: NON<ENR<SUP compared to animals in the other housing conditions.  (See Table 13a and Figures 17a - 17c.)

*Phase B – Standard Chow with Sweet and Salty Foods Period.*  The Kruskal-Wallis Chi-Square was not significant for number of animals moving, but was significant for amount of activity, and level of activity.  Kruskal-Wallis mean

rank orders indicated that all enrichment conditions had the same rank order with regard to the mean number of animals moving: NON=ENR=SUP, but that animals in the SUP condition moved the most: NON<ENR<SUP, and had the highest levels of activity: NON<ENR<SUP.  (See Table 13b and Figures 17a - 17c.)

*Phase A2 – Standard Chow Only Period.*  The Kruskal-Wallis test Chi-Square was not significant for number of animals moving, but was significant for amount of activity, and level of activity.  Kruskal-Wallis mean rank orders indicated that all enrichment conditions had the same rank order with regard to the mean number of animals moving: NON=ENR=SUP, but that animals in the SUP condition moved the most: NON<ENR<SUP, and had the highest levels of activity: NON<ENR<SUP.  (See Table 13c and Figures 17a - 17c.)

**Open Field (OF).**  A total of six OF trials examining horizontal, vertical, and center-time activity were accomplished during Experiment II (2 per experimental phase; A1, B, and A2).  Overall MANOVAs revealed significant differences between housing conditions in total horizontal activity or vertical activity (See Table 12a).  Therefore, the differences in horizontal and vertical activity were then examined using repeated-measures ANOVAs for each individual Open-Field trial.  The results of the between-groups and the within-session analyses are presented separately for each Open-Field trial.  Tables 14a – 14i present the details of the statistical analyses for the Open-Field trials.  Figures 18a - 18d present graphical depictions of the within-session horizontal and vertical activity for Open-Field trials.

*Phase A1 – Standard Chow Only Period, Open-Field Trial 1 Between*

*Groups.* There were significant differences for horizontal activity ($F_{(2,33)}$ =

21.539, $p < 0.05$) and vertical activity ($F_{(2,33)}$ = 13.313, $p < 0.05$). Pairwise

comparison post hoc analyses revealed that animals in the SUP condition moved

significantly less than the ENR and NON conditions. Further, there were no

significant differences between ENR and NON housed animals for horizontal and

vertical activity NON$\leq$ENR<SUP. (See Tables 14b & 14c and Figures 18a &

18b.)

*Phase A1 – Standard Chow Only Period, Within Groups Within Session.*

Repeated-measures ANOVAs revealed significant main effects for time on

horizontal activity ($F_{(11, 363)}$ = 63.488, $p < 0.05$) and vertical activity ($F_{(11,}$

$_{363)}$ = 48.803, $p < 0.05$). Animals' horizontal and vertical activity were different

over time. In addition, there was a significant time by housing interaction for

horizontal activity ($F_{(22, 363)}$ = 1.911, $p < 0.05$) and vertical activity ($F_{(22, 363)}$

= 2.029, $p < 0.05$). Post hoc analyses indicated that over time, SUP housed

animals moved the least, followed by the ENR, and the NON housed animals

had the highest amount of activity (NON<ENR<SUP). (See Tables 14b & 14c

and Figures 18a & 18b.)

*Phase A1 – Standard Chow Only Period, Open-Field Trial 2 Between*

*Groups.* Horizontal activity ($F_{(2,33)}$ = 47.870, $p < 0.05$) and vertical activity ($F$

$_{(2,33)}$ = 28.744 $p < 0.05$) were significantly different between housing conditions.

Pairwise comparisons indicated that SUP horizontal was significantly less than

ENR, which was significantly less than NON housing conditions

(NON>ENR>SUP). Animals in SUP conditions had significantly less vertical activity than did animals in the ENR and NON conditions, which were not significantly different from each other: NON$\geq$ENR>SUP. (See Tables 14d & 14e and Figures 18a & 18b.)

Phase A1 – Standard Chow Only Period, Open-Field Trial 2 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F$ (11, 363) = 49.272, $p$ < 0.05) and vertical activity ($F$ (11, 363) = 28.744, $p$ < 0.05), indicating that horizontal and vertical activity were different over time. In addition, there was a significant time by housing interaction for horizontal activity ($F$ (22, 363) = 1.863, $p$ < 0.05) and vertical activity ($F$ (22, 363) = 1.807, $p$ < 0.05). Post hoc analyses indicated that the decrease in activity over time was different for the three housing conditions such that the amount of activity decrease was most in the SUP followed by the ENR, and the NON housed animals had the lowest activity (NON<ENR<SUP). (See Tables 14d & 14e and Figures 18a & 18b.)

*Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 3 Between Groups.* Horizontal activity ($F$ (2,33) = 11.052, $p$ < 0.05) was significantly different between groups. Post-hoc analyses revealed that horizontal activity was lower for SUP compared to ENR and NON (NON$\geq$ENR>SUP). There were no significant differences in vertical activity. (See Tables 14f & 14g and Figures 18a & 18b.)

*Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 3 Within Groups Within Session.* Repeated-measures ANOVAs

revealed significant main effects for time on horizontal activity ($F$ (11, 363) = 44.923, $p$ < 0.05) and vertical activity ($F$ (11, 363) = 33.911, $p$ < 0.05), indicating that horizontal and vertical activity were different over time. A significant time by housing interaction for horizontal activity ($F$ (22, 363) = 1.170, $p$ < 0.05) suggests that animal horizontal activity decreased differently over time in all conditions. Post hoc analyses revealed that the decrease in horizontal activity over time was different between the three housing conditions such that activity decreased most in the SUP followed by the ENR, and the NON housed animals had the lowest amount of activity (NON<ENR<SUP). There was no significant time by housing interaction for vertical activity. (See Tables 14f & 14g and Figures 18a & 18b.)

   *Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 4 Between Groups.* Horizontal activity ($F$ (2,33) = 28.730, $p$ < 0.05) and vertical activity ($F$ (2,33) = 7.744, $p$ < 0.05) was significantly different between groups. Post Hoc Pairwise comparisons revealed lower activity for SUP and ENR compared to NON, but no significant difference between ENR and NON  (NON≥ENR>SUP). (See Tables 14h & 14i and Figures 18a & 18b.).

   Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 4 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F$ (11, 363) = 60.282, $p$ < 0.05) and vertical activity ($F$ (11, 363) = 32.367, $p$ < 0.05), indicating that horizontal and vertical activity were different over time. Significant time by housing interactions for horizontal activity ($F$ (22, 363) = 1.807, $p$ < 0.05) and

vertical activity ($F$ (22, 363) = 1.810, $p$ < 0.05) indicate that the difference in activity was different for the various housing groups. Post Hoc analyses revealed the decrease in activity over time was different for the three housing conditions such that the SUP housed animals moved the least, followed by the ENR, and the NON housed animals had the most activity (NON<ENR<SUP). (See Tables 14h &14i and Figures 18a & 18b.)

 *Phase A2 – Standard Chow Only Period, Open-Field Trial 5 Between Groups.* Horizontal activity ($F$ (2,33) = 25.648, $p$ < 0.05) and vertical activity ($F$ (2,33) = 12.984, $p$ < 0.05) were significantly different between groups. Post Hoc analyses revealed that horizontal and vertical activity were significantly lower animals in SUP housing compared to animals in the ENR, and animals in the ENR housing compared to animals in the NON housing (NON>ENR>SUP). (See Tables 14j & 14k and Figures 18a & 18b.)

 *Phase A2 – Standard Chow Only Period, Open-Field Trial 5 Within Groups Within Session.* Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F$ (11, 363) = 67.661, $p$ < 0.05) and vertical activity ($F$ (11, 363) = 42.938, $p$ < 0.05), indicating that animal activity decreased over time in all conditions. In addition, there was a significant time by housing interaction for horizontal activity ($F$ (22, 363) = 1.596, $p$ < 0.05) and vertical activity ($F$ (22, 363) = 1.695, $p$ < 0.05). Post Hoc analyses revealed that the over time, the amount of activity was least in the SUP followed by the ENR, and the NON housed animals had the most activity (NON<ENR<SUP). (See Tables 14j &14k and Figures 18a & 18b.)

*Phase A2 – Standard Chow Only Period, Open-Field Trial 6 Between*

*Groups.* Horizontal activity ($F_{(2,33)}$ = 7.528, $p$ < 0.05) and vertical activity ($F_{(2,33)}$ = 6.497, $p$ < 0.05) were significantly different between groups. Post Hoc Pairwise comparisons indicated lower activity for SUP housed animals compared to animals housed in the ENR and NON conditions. There were no significant differences between the animals in the ENR and NON housing conditions. For both horizontal and vertical activity: NON≥ENR>SUP. (See Tables 14l & 14m and Figures 18a & 18b.)

*Phase A2 – Standard Chow Only Period, Open-Field Trial 6 Within*

*Groups Within Session.* Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F_{(11, 363)}$ = 56.915, $p$ < 0.05) and vertical activity ($F_{(11, 363)}$ = 37.761, $p$ < 0.05), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity ($F_{(22, 363)}$ = 1.740, $p$ < 0.05) and vertical activity ($F_{(22, 363)}$ = 1.468, $p$ < 0.05) indicated that the decrease in activity over time was different for the three housing conditions. Post Hoc Pairwise comparisons revealed that the amount of activity was least in the SUP followed by the ENR, and the NON housed animals had the most activity: NON≥ENR>SUP. (See Tables 14l & 14m and Figures 18a & 18b.)

**Open-Field Results Summary.** Animals in the Enriched housing conditions were less active during each of the six OF trials. These animals had the greatest decrease in activity within each OF session compared with animals in the Non-Enriched housing conditions. (See Figures 18a – 18d.) In particular,

the animals in the Super-Enriched condition moved less than animals in the

Enriched condition, which moved less than animals in the Non-Enriched

condition. These differences in activity in the housing conditions are consistent

with other studies (Bowling, Rowlett, & Bardo, 1993; Van Waas & Soffie, 1996;

Varty et al., 2000; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001).

Further, there was a difference between all three housing conditions in the

amount of activity between OF trials such that the animals housed in the NON

condition moved the most and followed by ENR, and the SUP housed animals

moved the least. These effects were consistent with the findings from

Experiment I of this investigation.

### *Discussion: Experiment II*

Experiment II examined the effects of environmental enrichment on

activity, feeding, and body weight in female, adolescent, Sprague-Dawley rats.

Like Experiment I, this experiment was designed to examine the effects of

environmental enrichment on; (1) body weight when the different foods are

available, (2) food consumption, using bland foods and other, more preferred

foods, and (3) physical activity, including activity in an open field arena and within

the home cage.

The results of Experiment II indicated that animals housed in

environmental enrichment had significantly lower body weights. This finding is

consistent with Experiment I and with previous research (e.g., Sclafani &

Springer, 1976; Tomchesson, 2004). Additionally, animals housed in the Super-

Enrichment condition had significantly lower BMI and LI scores compared to the

Enriched and Non-Enriched conditions. These lower indices suggest that animals housed in enriched environments had lower amounts of body fat, particularly the SUP housed animals (Maffie et al., 1995). There were no signs of ill health in the animals that might account for the lower BW, BMI, or LI.

As in Experiment I, enrichment again decreased the amount of standard chow eaten early in the Experiment II. As before, the initial decreased feeding was consistent with lower body weights and body fat indices. However, during Phase B, the consumption of all foods again increased. There were no differences in the standard chow or potato chips eaten between housing groups. There was a significant difference in the amount of cookies eaten, with the Non-Enriched housed animals eating significantly more than the Enriched housed and Super-Enriched housed animals. In addition, during Phase A2, it appeared that the Super-Enriched began eating more bland food than both other groups, although this difference was not statistically significant.

Physical activity was measured in novel open field environments and in home cages. Open Field activity (horizontal and vertical activity) was lower for the animals housed in enrichment and was the lowest for the animals housed in the SUP condition. The effect for enrichment to decrease OF activity is consistent with Experiment I and previous reports (e.g., O'Conner & Eikelboom, 2000; Varty et al., 2000; Elliott 2004; Tomchesson, 2004). The decrease in OF activity is not consistent with lower body weights or body fat indices. Animals in the environmental enrichment were more active in their home cages, consistent with decreased body weight. Further, the home cage activity scores were

consistent with the differential decreases in body weight seen in the different housing conditions (i.e., BW = NON>ENR$\geq$SUP and HCA = NON<ENR$\leq$SUP).

Given the findings from Experiments I & II, it was important to attempt to replicate the decreased body weight and increased home cage activity in male and female subjects. In addition, it was also necessary to examine male and female subjects together to directly compare any gender differences. Interestingly, food consumption findings from this experiment suggest possible gender differences in the types of foods consumed by Enriched and Non-Enriched housed animals. Specifically, females in Non-Enriched housing ate significantly more cookies than did the females in the enriched conditions. This same difference did not reach significance for males, although there was a trend in this direction, in Experiment I.

**Experiment III**

Experiment III was conducted to attempt to replicate the findings of Experiments I and II and to allow a direct comparison of male and female subjects within the same experiment. The exact procedures of Experiment III were determined based on the results of the first two experiments. Experiment III was a 2 (housing condition) X 2 (gender) repeated-measures design investigating the effects of environmental enrichment on food consumption, physical activity, and body weight in male and female adolescent rats. The 7-week experiment used the same food types (sweet with bland, salty with bland, or bland only) and the housing conditions (NON, SUP) that had the greatest effects on body weight in the first two experiments. The findings from the FC

data regarding specific foods were unclear because in Experiment I, there was a trend for cookie consumption, but the difference was not statistically significant approaching significance. Conversely, the difference in cookie consumption among housing conditions was statistically significant in Experiment II. It is possible that there is a gender difference in the types of specific foods consumed, however, it is not possible to make a direct comparison, as these were two distinct and separate experiments. HCA data were clear but the measure was new and replicating the results is important to establish the reliability of the measure. The goals of Experiment III were to: (1) compare the effects of environmental enrichment on body weight in male and female adolescent rats, (2) compare the effects of environmental enrichment on food consumption in male and female adolescent rats, and (3) compare the effects of environmental enrichment on physical activity in male and female adolescent rats. The individual hypotheses for Experiment III were based on the results of Experiments I and II.

### *Hypotheses: Experiment III*

### *Hypothesis 1 – EE, BW, BMI, and LI*

Environmental enrichment will decrease body weight gains and body mass index such that: Non-Enriched > Super-Enriched.

### *Hypothesis 2 – EE and FC*

A) The animals in the Super-Enrichment condition will consume less bland food than Non-Enriched housing.

B) Males will consume more standard rat chow than females.

C) Males and females will consume more food during Phase B compared to Phase A1 & A2 regardless of housing condition.

D) There will be a sex by housing interaction for the type of foods consumed during Phase B for females such that animals reared in the Super-Enriched condition will eat less than animals in the Non-Enriched condition during Phase B.  However, there will be no significant differences in the amount of food consumed by males during Phase B.

### Hypothesis 3 – EE and PA

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Non-Enriched > Super-Enriched.

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Non-Enriched < Super-Enriched.

C) Males and females will exhibit different activity levels in both Open-Field trials and Home Cage observations such that males will be less active in the Open-Field, but more active in the home cage.

### Methods: Experiment III

The methods and design of Experiments I and II were used in Experiment III with three main differences.  First, both male and female subjects were used in an attempt to replicate the findings of the first two experiments and to allow for a direct gender comparison.  Second, as in Experiment II, only the group home cage activity (HCA) method was used.  Third, Open-Field (OF) activity was

measured three times at the end of each experimental phase and not six times at the beginning and end of each phase as in the previous experiments. This change was made because of the robust and consistent findings of previous experiments, including the first two experiments in this project.

### Subjects

Subjects were 48, adolescent (21 days old on arrival), male (24) and female (24) Sprague-Dawley rats.

### Housing

Housing conditions were identical to Experiments I & II with the exception that only the Non–Enriched and Super-Enriched housing conditions were used. The use of these two housing conditions was determined by the results of the first two experiments.  All animals were housed on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001 or "Junk" Foods) and water.  Two Super-Enriched housing cages were used in this experiment.  Each cage housed one sex of rats.

### Procedures

The procedures in Experiment III were virtually identical to Experiments I and II.  Experiment III was a 7-week, repeated-measures A-B-A design.  Unlike the first two experiments, it was a 2 (housing condition) X 2 (sex) repeated-measures design.  OF activity was measured only three times (not six) because the measure was so consistent that it proved to be redundant to measure it six times.  (See Table 15 for Experiment III Timeline.)

*Dependent Variables*

The dependent variables were identical to Experiment I & II.

**Body Weight (BW).** BW was measured on Day 1 and then three times during each 14-day experimental phase (the 5th, 10th, and 14th day) and two additional times for Phase A2 (the 18th and 21st day).

**Body Mass Index (BMI).** BMI was measured on the final day of the experiment.

**Lee Index (LI).** LI was measured on the final day of the experiment.

**Food Consumption (FC).** Food weights were measured every other day providing a total of 21 Food Consumption measurements.

**Physical Activity (PA).** *Home Cage Activity – Group Activity.* Group activity was observed a minimum of five times during experimental phase B and A2.

*Open-Field (OF).* OF activity was measured three times, once at the end of each experimental phase (Days, A1-13, B1-13, and A2-20).

## Results: Experiment III

**Body Weight (BW)**

Body weight was measured 12 times during the experiment (four times during Phase A1, three times during Phase B, and five times during the Phase A2). A between-subjects main effect for housing ($F$ (1, 42) = 5.578, $p < 0.05$) indicated that animals in the Non-Enriched housing weighed significantly more than animals in the Super-Enriched housing. A between-subjects main effect for sex ($F$ (1, 42) = 15.293, $p < 0.05$) revealed that the males weighed significantly

more than females.  Post Hoc pairwise comparisons revealed that males housed in Super-Enriched cages weighed significantly less than males housed in Non-Enriched cages, and females housed in Super-Enriched cages weighed significantly less than females in Non-Enriched housing (male NON > male SUP > female NON > female SUP).   A main effect for Time revealed that body weights differed over time ($F$ (10, 420) = 4.156, $p$ < 0.05).  A Time X Housing interaction ($F$ (10, 420) = 12.096, $p$ < 0.05) and a Time X Sex interaction ($F$ (10, 420) = 116.116, $p$ < 0.05) revealed that over time, animals weights differed when accounting for housing condition and sex.  Post Hoc pairwise comparisons revealed that animals in the NON housing gained weight faster than animals in the SUP housing and males gained weight faster than females.  There were no significant differences in body length between the housing conditions.  There was a significant difference in body length between males and females, as would be expected given the difference in sizes between the males and females, and there was not a gender by housing interaction (See Table 22).  (See Table 16 and Figures 19a -19c. for body weight results)

### Body Mass Index (BMI)

Animals housed in Super-Enriched housing had significantly different BMIs compared to animals housed in Non-Enriched housing ($F$ (1,43) = 15.458, $p$ < 0.05).  Females had significantly different BMI than males ($F$ (1,43) = 14.674, $p$ < 0.05).  There was no housing X sex interaction.  Tukey HSD post hoc analyses revealed a strong trend for animals in the Super-Enriched Females to have significantly lower BMIs compared to Non-Enriched Females.  Super-

Enriched Males had significantly lower BMIs than Non-Enriched Males (NON

Males> NON Females $\geq$ SUP Males $\geq$ SUP Females).  (See Table 17 and Figure

20.)

### Lee Index (LI)

Animals in Super-Enriched housing had significantly lower LIs compared

to animals housed in Non-Enriched housing ($\underline{F}$ (1,43) = 8.190, $\underline{p}$ < 0.05).  There

was no main effect for sex, nor a sex X housing interaction.  (NON Females $\geq$

NON Males $\geq$ SUP Females $\geq$ SUP Males).  (See Table 18 and Figure 21.)

### Food Consumption (FC)

Food consumption (FC) was measured 25 times during Experiment I

(seven measurements for Phase A1 & B and 11 measurements for Phase A2).

Average grams and calories consumed daily were examined separately for each

experimental phase because the available foods differed for each phase (i.e.,

during Phases A1 & A2, only standard chow was available and during Phase B,

standard chow, salty, and sweet foods were available).  The results for grams

and calories are presented separately for each phase of the experiment.

*Phase A1 – Standard Chow Only Period: GRAMS.*  Animals in the SUP

housing ate significantly less than animals housed in the NON housing ($\underline{F}$ (1, 43)

= 69.175, $\underline{p}$ < 0.05) and females ate significantly less than males ($\underline{F}$ (1, 43) =

43.469, $\underline{p}$ < 0.05).  A Tukey HSD comparison revealed Super-Enriched females

ate significantly less than Super-Enriched males and Non-Enriched females that

ate significantly less than Non-Enriched males  (NON Males > NON Females $\geq$

SUP Males > SUP Females).  (See Table 19a and Figure 22a.)

***Phase A1 – Standard Chow Only Period: CALORIES.*** Animals in the SUP housing ate significantly fewer calories than animals housed in the NON housing ($F$ (1, 43) = 69.175, $p$ < 0.05) and females ate significantly fewer calories than males ($F$ (1, 43) = 43.469, $p$ < 0.05). A Tukey HSD comparison revealed Super-Enriched females ate significantly less than Super-Enriched males and Non-Enriched females who ate significantly less than Non-Enriched males  (NON Males > NON Females $\geq$ SUP Males > SUP Females).  (See Table 19b and Figure 22b.)

***Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: GRAMS.*** Animals in the SUP housing ate significantly less than animals housed in the NON housing ($F$ (1, 43) = 40.851, $p$ < 0.05) and females ate significantly less than males ($F$ (1, 43) = 4.342, $p$ < 0.05). A Tukey HSD comparison revealed Super-Enriched females ate significantly less than Non-Enriched females, that ate similar amounts of chow compared to Super-Enriched males, that ate significantly less than Non-Enriched males (NON Males $\geq$ SUP Males $\geq$ NON Females > SUP Females). (See Table 19c and Figure 22a.)

***Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: CALORIES.*** Animals in the SUP housing ate significantly fewer calories than animals housed in the NON housing ($F$ (1, 43) = 40.851, $p$ < 0.05) and females ate significantly fewer calories than males ($F$ (1, 43) = 4.342, $p$ < 0.05). A Tukey HSD comparison revealed Super-Enriched females ate only slightly fewer calories than Super-Enriched males, who ate significantly fewer calories than Non-Enriched females, who ate significantly fewer than Non-Enriched males

(NON Males > NON Females > SUP Males $\geq$ SUP Females).  (See Table 19d and Figure 22b.)

Specific Food Types examined separately:

***Phase B – Standard Chow: GRAMS.***  Animals in the SUP housing did not eat significantly less than animals housed in the NON housing, however, females ate significantly less than males ($F$ (1, 43) = 150.316, $p$ < 0.05).  In addition, a significant housing by sex interaction ($F$ (1, 43) = 4.999, $p$ < 0.05) revealed that females housed in the SUP condition ate less standard chow than did females in the NON condition, but males in the SUP ate more standard chow than did NON housed males.  A Tukey HSD comparison revealed that Super-Enriched females ate slightly less standard chow than Non-Enriched females, that ate significantly less than Super-Enriched males, that ate marginally less than Non-Enriched males (SUP Males $\geq$ NON Males > NON Females $\geq$ SUP Females).  (See Table 19e and Figure 22c.)

***Phase B – Standard Chow: CALORIES.***  Animals in the SUP housing did not eat significantly less than animals housed in the NON housing; however, females ate significantly less than males ($F$ (1, 43) = 150.316, $p$ < 0.05).  In addition, a significant housing by sex interaction ($F$ (1, 43) = 4.999, $p$ < 0.05) revealed that female animals housed in the SUP condition ate less standard chow than did females in the NON condition, but males in the SUP ate more standard chow than did NON housed males.  A Tukey HSD comparison revealed that Super-Enriched females ate slightly less standard chow than Non-Enriched females, that ate significantly less than Super-Enriched males, that ate

marginally less than Non-Enriched males (SUP Males $\geq$ NON Males > NON Females $\geq$ SUP Females).  (See Table 19f and Figure 22d.)

*Phase B – Cookies: GRAMS*. Animals in the SUP housing ate significantly less than animals housed in the NON housing ($F$ (1, 43) = 17.454, $p$ < 0.05) and there was no difference in the amount of cookies consumed between males and females.  A Tukey HSD comparison revealed Super-Enriched males ate slightly less cookies than Super-Enriched females, that ate less than Non-Enriched males, that ate less than Non-Enriched females (NON Females $\geq$ NON Males $\geq$ SUP Females $\geq$ SUP Males).   (See Table 19g and Figure 22c.)

*Phase B – Cookies: CALORIES*. Animals in the SUP housing ate significantly fewer calories than animals housed in the NON housing ($F$ (1, 43) = 25.938, $p$ < 0.05) and there was no difference in the cookie calories consumed between males and females.  A Tukey HSD comparison revealed that Super-Enriched males ate slightly less cookies than Super-Enriched females, that ate less than Non-Enriched males, that ate less than Non-Enriched females (NON Females $\geq$ NON Males $\geq$ SUP Females $\geq$ SUP Males).  (See Table 19h and Figure 22d.)

*Phase B – Potato Chip: GRAMS.*  Animals in the SUP housing ate significantly fewer potato chips than animals housed in the NON housing ($F$ (1, 43) = 21.237, $p$ < 0.05) and there was no difference in the amount of cookies consumed between males and females.  A Tukey HSD comparison revealed that Super-Enriched males ate slightly fewer cookies than Super-Enriched females, that ate less than Non-Enriched males, that ate less than Non-Enriched females

(NON Males $\geq$ NON Females > SUP Males $\geq$ SUP Females).  (See Table 19i and Figure 22c.)

     ***Phase B – Potato Chip: CALORIES***.  Animals in the SUP housing ate significantly fewer chip calories than animals housed in the NON housing ($F$ (1, 43) = 16.437, $p$ < 0.05) and males consumed more chip calories than females ($F$ (1, 43) = 4.333, $p$ < 0.05).  A Tukey HSD comparison revealed that Super-Enriched males ate less cookies than Super-Enriched females, that ate less than Non-Enriched males, that ate less than Non-Enriched females (NON Males $\geq$ NON Females > SUP Males $\geq$ SUP Females).  (See Table 26 and Figure 20d.)

     ***Phase A2 – Standard Chow Only Period: GRAMS***.  Animals in the SUP housing ate significantly ate more than animals housed in the NON housing ($F$ (1, 43) = 59.788, $p$ < 0.05) and males ate more than females ($F$ (1, 43) = 294.360, $p$ < 0.05).  A Tukey HSD comparison revealed that Super-Enriched males ate significantly less cookies than Super-Enriched females, that ate significantly less than Non-Enriched males, that ate significantly less than Non-Enriched females (NON Males > NON Females > SUP Males > SUP Females). (See Table 19j and Figure 22a.)

     ***Phase A2 – Standard Chow Only Period: CALORIES***.  Animals in the SUP housing ate significantly more than animals housed in the NON housing ($F$ (1, 43) = 59.788, $p$ < 0.05) and males ate more than females ($F$ (1, 43) = 294.360, $p$ < 0.05).  A Tukey HSD comparison revealed that Super-Enriched males ate significantly fewer calories than Super-Enriched females, that ate significantly fewer calories than Non-Enriched males, that ate significantly fewer

calories than Non-Enriched females (NON Males > NON Females > SUP Males > SUP Females).  (See Table 19l and Figure 22b.)

**Physical Activity (PA)**

    ***Home Cage Activity (HCA).***  HCA was measured 17 times during Experiment I (six times during Phase B and 11 times during Phase A2).  The average number of animals moving, amount of activity, and level of activity were observed during each measurement.  Each type of HCA is presented for each experimental phase.

    <u>Phase A1 – Standard Chow only Period</u>.  The Kruskal-Wallis Chi-Square was not significant for number of animals moving, but was significant for amount of activity, and level of activity.  Kruskal-Wallis mean rank orders indicated that all enrichment conditions were similar with regard to the mean number of animals moving: NON Females $\leq$ NON Males $\leq$ SUP Males $\leq$ SUP Females.  Animals in the SUP conditions had the most activity, with Super-Enriched males ranked as the most active and Non-Enriched females as the least active: NON Females < NON Males < SUP Females < SUP Males.  Super-Enriched males had the highest level of activity and Non-Enriched Females had the lowest rank for level of activity: NON Females < NON Males < SUP Females < SUP Males.  (See Table 20a and Figures 23a - 23c.)

    <u>Phase B – Standard Chow with Sweet and Salty Foods Period</u>.  The Kruskal-Wallis Chi-Square was significant for number of animals moving, amount of activity, and level of activity.  Kruskal-Wallis mean rank orders indicated that three conditions were similar with regard to the mean number of animals moving:

NON Females = SUP Males = SUP Females > NON Males.  Animals in the SUP conditions had the most activity, with Super-Enriched Females ranked as the most active and Non-Enriched Females as the least active: NON Females < NON Males < SUP Males < SUP Females.  Super-Enriched Females had the highest level of activity and Non-Enriched Males had the lowest rank for level of activity: NON Males < NON Females < SUP Males < SUP Females.  (See Table 20b and Figures 23a - 23c.)

   *Phase A2 – Standard Chow Only Period.*  The Kruskal-Wallis Chi-Square was not significant for number of animals moving, but was significant for amount of activity, and level of activity.  Kruskal-Wallis mean rank orders indicated that all enrichment conditions were similar with regard to the mean number of animals moving: NON Females $\leq$ NON Males $\leq$ SUP Males $\leq$ SUP Females.  Animals in the SUP conditions had the most activity, with Super-Enriched females ranked as the most active and Non-Enriched females as the least active:  NON Females < NON Males < SUP Males < SUP Females.  Super-Enriched females had the highest level of activity and Non-Enriched Females had the lowest rank for level of activity: SUP Females < SUP Males < NON Males < NON Females.  (See Table 20c and Figures 23a - 23c.)

   **Open Field (OF).**  A total of three OF trials examining horizontal and vertical activity were accomplished during Experiment III (1 per experimental phase - A1, B, and A2).  Overall MANOVAs revealed that males and females exhibited similar total horizontal activity and vertical activity.  Super-Enriched housed animals displayed significantly less total horizontal and vertical activity

than did the Non-Enriched housed animals.  The results of the between-groups

and the within-session analyses are presented separately for each Open-Field

trial.  Tables 21a - 21d present the details of the statistical analyses for the

Open-Field trials.  Figures 24a - 24c present graphical depictions of the

horizontal and vertical activity for Open-Field trials.  (Note: Males displayed

significantly less vertical Activity during OF 3 which resulted in significant Sex X

Housing interaction because OF 3 was the only time the difference was

statistically significant.  Despite this effect, OF activity levels between males and

females were more similar than different.)

*Phase A1 – Standard Chow Only Period, Open-Field Trial 1 Between*

*Groups.*  Horizontal activity ($F$ (1,43) = 242.947, $p$ < 0.05) and vertical activity ($F$

(1,43) = 118.804, $p$ < 0.05) were significantly lower for animals housed in Super-

Enrichment compared to animals housed in the Non-Enriched condition.  There

were no significant differences between males and females or Sex X Housing

interactions.  (See Table 21a & 21b and Figures 24a & 24b.)

*Phase A1 – Standard Chow Only Period, Within Groups Within Session.*

Repeated-measures ANOVAs revealed significant main effects for time on

horizontal activity for vertical activity ($F$ (11,473) = 78.060, $p$ < 0.05) and vertical

activity ($F$ (11,473) = 58.025, $p$ < 0.05), indicating that animal activity decreased

over time in all conditions.  In addition, a significant time by housing interaction

for horizontal activity ($F$ (22, 363) = 21.548, $p$ < 0.05) and vertical activity ($F$ (22,

363) = 10.430, $p$ < 0.05) indicated that the decrease in activity over time was

different for the three housing conditions such that the activity decreased most in

the Super-Enriched compared to the Non-Enriched  housed animals.  There was no Time X Sex interaction revealing that between the sexes there was no difference in the decreased activity over time.  (See Table 21c & 21d and Figures 24c & 24d.)

*Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 2 Between Groups.*  Horizontal activity ($F$ (1,43) = 97.225, $p$ < 0.05) and vertical activity ($F$ ($F$ (1,43) = 45.398, $p$ < 0.05) were significantly lower for animals in the SUP housing compared to animals in the NON housing.  There was no difference in horizontal or vertical activity between males and females.  There was no Sex X Housing interaction suggesting that activity was consistent between males and females and between housing conditions or that there was insufficient power to detect any interactions between Sex and Housing.  (See Table 21a & 21b and Figures 24a & 24b.)

*Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 2 Within Groups Within Session.*  Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F$ (11, 473) = 65.474, $p$ < 0.05) and vertical activity ($F$ (11, 473) = 53.879, $p$ < 0.05), indicating that animal activity decreased over time in all conditions.  In addition, a significant time by housing interaction for horizontal activity ($F$ (11, 473) = 8.318, $p$ < 0.05) and vertical activity ($F$ (11, 473) = 5.164, $p$ < 0.05) indicated that over time, activity was greater in the NON housed animals compared to the SUP housed animals.  (See Table 21c & 21d and Figures 24c & 24d.)

*Phase A2 – Standard Chow Only Period, Open-Field Trial 3 Between*

*Groups.* Horizontal activity ($F_{(1,43)}$ = 87.960, $p < 0.05$) and vertical activity ($F_{(1,4\ 3)}$ = 71.806, $p < 0.05$) were significantly lower for SUP compared to NON. Males moved more vertically than did females ($F_{(1,43)}$ = 4.083, $p < 0.05$). There was a Sex X Housing interaction for vertical activity ($F_{(1,43)}$ = 5.141, $p < 0.05$). (See Table 21a & 21b and Figures 24a & 24b.)

*Phase A2 – Standard Chow Only Period, Open-Field Trial 3 Within*

*Groups Within Session.* Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($F_{(11, 473)}$ = 63.529, $p < 0.05$) and vertical activity ($F_{(11, 363)}$ = 37.862, $p < 0.05$), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity ($F_{(22, 363)}$ = 6.156, $p < 0.05$) and vertical activity ($F_{(22, 363)}$ = 2.396, $p < 0.05$) indicated that the decrease in activity over time was different between the three housing conditions such that the amount of activity decrease was greatest in the SUP compared to the NON housed animals. (See Table 21c & 21d and Figures 24c & 24d.)

**Open-Field Results Summary.** Animals in the Super-Enriched housing condition were less active during each of the three OF trials and these animals decreased activity more within each OF session compared with animals in the Non-Enriched housing conditions. These differences in activity in the housing conditions are consistent with the first two experiments in this series, and in other studies (Bowling, Rowlett, & Bardo, 1993; Van Waas & Soffie, 1996; Varty et al*.,*

2000; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001). (See Figures 24a - 24d.)

### *Discussion: Experiment III*

Experiment III was designed to replicate the findings from Experiments I & II, which examined the effects of environmental enrichment on: (1) body weight, when the different foods are available (2) food consumption, using bland foods and other, more preferred, foods, and (3) physical activity, including activity in an open field arena and within the home cage. In addition, Experiment III also directly compared these findings between male and female subjects.

The results of Experiment III indicated that animals housed in environmental enrichment had significantly lower body weights than animals not housed in enrichment. This finding is consistent with the first two experiments and with previous research (e.g., Sclafani & Springer, 1976; Tomchesson, 2004). Males had greater body weights than females. Animals housed in the Super-Enrichment had significantly lower BMI and LI scores compared to the Non-Enriched conditions. Males had significantly greater BMI scores than females. The lower indices suggest that animals housed in enriched environments had lower amounts of body fat (Maffie et al., 1995). There was no indication of poor health for the animals during Experiment III that could account for the lower BW, BMI, and LI.

Standard chow consumption was lower in the Super-enrichment condition. The decreased feeding again disappeared, as sweet and salty foods were made available. During this time, enrichment's effect to decrease body weight

appeared to be attenuated.  With regard to the previous findings for specific foods during Phase B, many of the findings for different types of foods were not replicated.  For example, males clearly preferred cookies to chips during Experiment I, whereas in Experiment III, males ate more chips than cookies.  Also, the amount of standard chow was nearly identical for both males and females in the first two experiments, but in Experiment III Super-Enriched animals ate significantly less standard chow than the Non-Enriched housed animals for both males and females.  Importantly, animals ate more of the preferred foods regardless of housing condition.  Interestingly, the females ate more cookies than did the males (this finding was noted in the data from the first two experiments, but could not be directly compared).  This finding is interesting because the males consumed more food in weight and calories for all foods except for cookies, which females consumed more than males.

Physical activity was measured in novel open field (OF) and home cages (HCA).  OF activity (horizontal and vertical activity) was lower for the animals housed in enrichment.  Males and females did not differ in the amount of their OF activity.  Again, this decrease in OF activity is not consistent with a decrease in body weight but home cage activity results were consistent with lower body weights.  For HCA, Super-Enriched housed animals moved more, had higher levels of activity, and weighed less than the animals in the Non-Enriched housing.  Females had higher amounts and levels of activity compared to the males, which may help to explain why males seem to gain weight more rapidly than females.

In summary, Experiment III replicated several of the major findings from the first two experiments. Animals housed in environmental enrichment weighed less than animals in Non-Enriched housing, had lower BMI and LI scores, were more active in the OF environment, and were more active in their home cages. Food consumption was different for the specific types of foods; however, all animals ate more when more preferred foods were available, regardless of housing condition. In general, males and females appear to respond similarly to environmental enrichment.

## SECTION III - ASSESSMENT & DISCUSSION

### Assessment of Experimental Hypotheses

### *Hypotheses: Experiment I*

### *Hypothesis 1 – EE, BW, BMI, and LI*

Environmental enrichment will decrease body weight gains and body mass index such that: Super-Enriched < Enriched < Non-Enriched. ***Partially Confirmed -*** the rank order of the housing conditions by mean body weight, BMI, or LI were as predicted, however, only the Non-Enriched and Super-Enriched conditions was statistically different from each other for body weight. For LI and BMI, Super-Enriched and Enriched were not statistically different from each other, but were different from the Non-Enriched condition.

### *Hypothesis 2 – EE and FC*

A) Environmental enrichment will decrease the amount of bland foods consumed compared to animals reared in Non-Enrichment such that: Super-Enriched < Enriched < Non-Enriched. ***Partially Confirmed -*** the rank order of

the housing conditions by the mean amount of food consumed was as predicted, however, Super-Enriched males ate significantly less than Enriched and Non-Enriched males. The difference between Enriched and Non-Enriched animals was not statistically different.

B) Animals raised in enriched and non-enriched housing conditions will eat similar amounts of cafeteria foods such that: Super-Enriched = Enriched = Non-Enriched. ***Confirmed***

*Hypothesis 3 – EE and PA*

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Super-Enriched < Enriched < Non-Enriched. ***Confirmed***

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Super-Enriched > Enriched > Non-Enriched. ***Confirmed***

*Hypotheses: Experiment II*

*Hypothesis 1 – EE, BW, BMI, and LI*

Environmental enrichment will decrease body weight gains and body mass index, and Lee index such that: Super-Enriched < Enriched < Non-Enriched. ***Partially Confirmed -*** the rank order of the housing conditions by mean body weight, BMI, or LI was as predicted, however, the Non-Enriched and Enriched conditions were not statistically different from each other, but were statistically different from the Super-Enriched housing condition.

***Hypothesis 2 – EE and FC***

A) Environmental enrichment will decrease the amount of bland foods consumed compared to animals reared in Non-Enrichment such that:  Super-Enriched < Enriched < Non-Enriched.  ***Confirmed***

B) Animals raised in enriched and non-enriched housing conditions will eat similar amounts of cafeteria foods such that:  Super-Enriched = Enriched = Non-Enriched.  ***Partially Confirmed -*** Super-Enriched females ate significantly fewer Oreos than Enriched females, that ate significantly fewer Oreo cookies than Non-Enriched females which caused a significant difference in the total food consumed.  There were no differences in the amount of chips or standard foods consumed during Phase B.

***Hypothesis 3 – EE and PA***

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Super-Enriched < Enriched < Non-Enriched.  ***Confirmed***

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Super-Enriched > Enriched > Non-Enriched.  ***Confirmed***

***Hypotheses: Experiment III***

***Hypothesis 1 – EE, BW, BMI and LI***

Environmental enrichment will decrease body weight gains and body mass index such that: Super-Enriched < Non-Enriched. ***Confirmed***

*Hypothesis 2 – EE and FC*

A) The animals in the Super-Enrichment will consume less bland food than Non-Enriched housing.  ***Confirmed***

B) Males will consume more standard rat chow than females. ***Confirmed***

C) Males and females will consume more food during Phase B compared to Phase A1 & A2 regardless of housing condition.  ***Confirmed***

D) There will be a sex by housing interaction for the type of foods consumed during Phase B for females such that animals reared in the Super-Enriched condition will eat less than animals in the Non-Enriched condition during Phase B.  However, there will be no significant differences in the amount of food consumed by males during Phase B.  ***Not Confirmed -*** there were no Sex X Housing interactions for the amount of different types of foods consumed during Phase B.

*Hypothesis 3 – EE and PA*

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Super-Enriched < Non-Enriched.  ***Confirmed***

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Super-Enriched > Non-Enriched.  ***Confirmed***

C) Males and females will exhibit different activity levels in both Open-Field trials and Home Cage observations such that males will be less active in the Open-Field, but more active in the home cage.  ***Not Confirmed -*** there were

no significant sex differences in OF activity levels and Super-Enriched females appeared to be the most active animals and Non-Enriched females appeared to be the overall least active animals.

## General Discussion

Three separate experiments were conducted to examine the effects of environmental enrichment on body weight, feeding, and physical activity in male and female adolescent rats.  This project highlights three key findings that have been well established in the research literature.  First, rodent research models have proven to be a valuable means to conduct scientific research to inform the human condition in a variety of fields such as pharmacology, neuroscience, and psychology.  For example, the common first line of experimental trials in pharmacological research is typically a rodent (mouse or rat) investigation.  Ground-breaking research on psychological principles were conducted by such pioneers as Ivan Pavlov, B.F. Skinner, John Garcia, and Neal Miller.  Animal models allow for more experimental control and more precise determination of causality.  Interpretation and inference is used to extrapolate findings from animal experiments to humans.  It is with this goal in mind that the present investigation was conducted and findings may be extrapolated to humans.  The second major finding previously established in the research literature is that food consumption and physical activity are key factors that affect body weight (e.g. Premack & Premack, 1963; Sclafani & Springer, 1976; Sclafani & Gorman, 1977; Warwick, Synowski, & Bell, 2002; Lattanzio & Eikelboom, 2003).  This particular project again confirms that energy intake, in the form of food consumption, and energy

expenditure, in the form of physical activity, are important factors in regulating body weight. The third previously established finding is that sweet and salty foods are preferred over bland foods. Research supporting this fact has been well established for many years as illustrated by the seminal research of Sclafani and Springer (1976).

This research project adds four major findings to the research literature. First, environmental enrichment is an important factor that can influence food consumption, physical activity levels, and body weight. Although other research has reported findings that may be consistent with environmental influences on these factors, to this investigator's knowledge, none has specifically investigated environmental enrichment's effects on all of these factors. The finding that environmental enrichment can decrease body weight through energy intake and energy expenditure is noteworthy. Providing an environment with both social and physical stimuli might increase the activity levels of the individual and decrease the consumption of bland foods, thereby controlling body.

The second important finding from this investigation is that environmental enrichment may not be a strong enough influence to offset food consumption of preferred foods. Understanding that more preferred foods may be a stronger reinforcer than having social and physical stimuli available in the environment is critical in our understanding of weight management. If available, preferred foods will be eaten, regardless of the availability for activity or engaging in activity. Also, the potency of the preferred foods offers another interesting finding. It is clear that despite housing condition, food consumption increased dramatically

when more preferred foods were available.  In the group situations, the animals typically ate less preferred foods did than the isolated animals.  The animals in both groups ate more than they did when standard bland chow alone was available.

The third major finding from this project is that male and female rats appear to respond similarly to environmental enrichment with regard to body weight, feeding, and physical activity.  No other study has reported environmental enrichment's effects to decrease body weight, decrease feeding, decrease physical activity in an open field, and increase physical activity in the home cage in both adolescent male and female rats when compared to rats in a non-enriched housing condition.  Although most of the major findings from Experiments I & II were replicated, there was some discrepancy in the details regarding the specific food types that were preferred by adolescent males and females.  While the general replications suggest robust effects for enrichment's overall influence to decrease food consumption, further research is warranted before rendering any definitive conclusions regarding gender effects on the consumption of specific food types.  Despite this inconsistency, there were no major gender differences in how adolescent male and female Sprague-Dawley rats' feeding, activity, and body weight were affected by environmental enrichment.

The fourth major finding from this project is the introduction of a new critical variable in environmental enrichment research, home cage activity. Previously, no other research reported on how environmental enrichment affects

animal activity in their home cages.  In fact, the only activity reported in the literature was in a novel environment (i.e., Open-Field), which was not consistent with environmental enrichment's effect to decrease body weight.  This finding is important for two main reasons.  From a practical point of view, researchers need to be aware that providing social and physical stimuli into an animal's environment can introduce potential confounds for investigations, even for studies that are not examining environmental enrichment.  It is commonplace for researchers to include environmental enrichment as a standard method to house animals during experiments regardless of their research question.  Taken from Yerkes and Zoology, it is believed that enrichment is a more "humane" way of housing a captive animal.  It is important to know that housing animals in larger groups or in bigger cages may affect body weight, daily physical activity, and feeding.  Each of these factors potentially affects health, body composition, and metabolism, all of which are common dependent variables for a wide variety of investigations that have nothing to do with environmental enrichment.  For example, an investigation examining a novel drug compound that houses animals in enrichment may lead to a different conclusion regarding body weight than would be reached if all animals were housed in isolation.  The second reason home cage activity is an important introduction is that when extrapolating these findings to humans, it is important to provide more opportunities for social and physical stimuli in a child's environment to help manage their weight.

It is noteworthy that the Super-Enriched (SUP) condition used in this project probably is more similar to the natural environment in which rats live than

is the Non-Enriched (NON) condition. Therefore, the results of the present research may be regarded as the effects of "more natural" conditions to decrease body weight and increase home cage activity. However, the results may be regarded as the effects of "less natural" conditions to increase body weight and decrease home cage activity.

It also is important to note that the present research studied adolescent rats that became young adults over the course of the studies. Age affects body weight, food consumption, and physical activity. Therefore, the present findings apply to adolescent and young adult rats.

### *Potential Clinical Applications*

There are several potential clinical applications of this project's findings. The most basic application is eliminating access to unhealthy, great tasting, high caloric foods for adolescents and young adults attempting to control body weight. Many behavioral interventions (e.g., Thomas, 1995; Behavioral Choice Treatment, Sbrocco, et al., 1999, personal communication 2004; Treasure et al., 2003) suggest moderation and do not support the "complete elimination" of high caloric snacks. While moderation may be a more "acceptable" approach for adolescents and young adults concerned with weight loss, this project's findings suggest that when high caloric, preferred foods are available, they are going to be eaten in excess. Therefore, such foods should not be available. Further, portion sizes should be monitored and controlled. Any excess food left over after the snack should be discarded because, according to the findings of this study, if the food is available it will be eaten. In line with this recommendation, snack

machines in military dorms and schools might limit the availability of unhealthy, high caloric, junk foods.  An additional benefit of this intervention also may be the increase in physical activity (i.e., energy expenditure) required to "seek out" the high caloric foods.  The practice of eliminating cues that may signal eating (e.g., junk food) is called stimulus control (i.e., using a tangible object or other cue as a reminder for a given behavior).

In many weight loss interventions, stimulus control is not simply getting rid of food, there are other ways to use stimulus control to limit food intake.  For example, eating only at the dining room table, eating only at specific times of day, or not eating while watching TV.  Given the findings from this study, providing the opportunities for activity becomes the stimulus control.  This can be accomplished by providing bicycles to ride, stationary bikes in front of televisions or video games, posters of exercising, set times to exercise or engage in activity at home.   Within the home environment, it may take great effort to provide opportunities to be more active.  Providing balls or other exercise equipment, jump ropes, hula-hoops, or trampolines at home can encourage activity.

Ironically, findings from these investigations of animal behavior may be applied to cognitive interventions for weight management.  As part of a multi-modal intervention for weight management, thought monitoring and challenging negative thoughts regarding one's weight are standard interventions.  It is generally accepted that people do not respond uniformly to therapeutic approaches.  Consequently, adapting monothetic therapeutic interventions to each individual is typically recommended.  As such, a thorough functional

analysis is important to guide the thought challenging and the specific thoughts to be challenged can be quite different from individual to individual. For example, the thought "I can't lose weight" requires gathering evidence about one's ability to lose weight. Conversely, the thought "I am too big to exercise" requires gathering evidence to support that "big people" can exercise, too. Each of these thoughts leads to different problem solving approaches. The results of this project suggest that interventions should also focus on the thoughts related to one's ability to alter their environment (e.g., by including physical and social stimuli conducive to activity). In addition, thoughts regarding the ability to substitute more healthy foods into their daily schedule, go shopping, would also be important to include.

The most important clinical application is the provision of physical and social stimuli to promote activity in home, school, and work environments (wherever individuals spend the most time). Providing the opportunity to become more active is an important job for parents at home but also for the school systems because children spend a large portion of their day in school. Currently, many elementary, middle, and high schools have severely limited opportunities for physical activity and have inadequate physical education programs. Results of this study indicate that by providing a natural environment suited to physical activity and social stimulation (i.e., enrichment), children might be more likely to be more active, resulting in lower body weights. In addition to increasing the frequency, duration, and intensity of physical education classes, adding physical activity inside the classroom may be warranted. For example, incorporating

learning games requiring energy expenditure, such as standing and walking around the classroom, dancing in place, and limited exercise movements (e.g., stretching) might help increase activity.

### *Limitations and Future Directions*

No research is perfect or without its limitations. Also, no research can examine every possible aspect of a given question. This project used only adolescent animals. The principal focus was on adolescents because of the current weight epidemic in children, but the findings of this study are limited to adolescents. Further investigation would be needed to determine whether the findings from this project generalize to additional age groups. This study used of only one strain of rat. The use of multiple strains could provide greater generalizability of findings across a more diverse population. The use of the Sprague-Dawley strain, the most widely used strain, allows for the greatest generalizability while using only one strain, but the use of multiple strains could provide greater utility to the findings should they replicate in the additional strains. Physical and social stimuli were used concurrently and not separated in this study. It is impossible to determine if a social or physical component is more important in enrichment's effects on body weight, feeding, and physical activity. For example, it is not clear if simply being around other animals led to an increase in home cage activity or if just having more room in the cage led to an increase in physical activity. This distinction could have important implications in how enrichment might be used to help manage an individual's body weight. This investigation did not examine metabolic activity. While it was beyond the scope

of this project, it is possible that changes that occur within the body in response to environmental enrichment cause alter metabolism. This project focused only on rats and did not use human subjects. In addition, the A-B-A design may have introduced time effects and order effects. The inclusion of B-A-B design or the use of a strictly between-subjects design would address these possibilities.

Several possible directions for future research could be explored. It would be valuable to examine the effect of environmental enrichment in different age groups and in multiple strains of rats to increase the generalizability of the findings. By separating social and physical components of environmental enrichment, important information may be obtained about how best to employ enrichment as a weight management strategy. More intriguing would be to conduct a human parallel study to examine a group of diverse individuals in a confined environment and see how they respond to environmental enrichment (e.g., as a member of the military, on a military installation during training, or during a remote assignment or deployment setting where the individuals are confined to a location for a given amount of time). It would be interesting to see how manipulating the environment to allow for increased or decreased social and physical stimulation impacts feeding, activity, and body weight.

It also would be valuable to examine possible explanations for the present results. For example, the SUP housing conditions provided increased floor space and opportunities for movement; increased numbers of social interactions; and probably altered brain morphology such as cortical thickness and increased dendritic branching (based on archived research). Future experiments should

separate and examine these possible mechanisms or mediators of the housing-induced effects on body weight, food consumption, and physical activity.

### *Summary and Conclusions*

Providing opportunities for organisms to thrive and excel (i.e., environmental enrichment) has long-lasting positive biological and behavioral consequences such as increasing brain size, improving information processing and learning.  In addition, enriched environments can affect physical activity and feeding, two factors that can increase or decrease body weight.  Body weight is a concern because excessive body weight increases the risk for premature death and chronic illness.  Three separate experiments were conducted to examine the effects of environmental enrichment on body weight, feeding, and physical activity in male and female adolescent rats.

From this research project it is clear that environmental conditions, particularly housing conditions, are important in regulating body weight for adolescent male and female Sprague-Dawley rats.  Body weight is significantly reduced, the consumption of bland foods is reduced, and providing physical and social stimuli in the home cage environment increases activity in that cage.  The decrease in body weight may be accomplished through a decreased feeding that occurs when bland foods are available or by an increase in daily activity that seems to occur in the enriched environments.  This project provides evidence for the importance for considering animals' home cage activity and the use of environmental enrichment in animal research.  These findings also provide evidence for some of the clinical interventions already used in managing weight

loss or gain (e.g., stimulus control, social support).  Additionally, these findings suggest that increasing opportunities for social and physical interactions within the home environment is a potential means to improve weight management interventions for adolescent and young adult humans.

# SECTION IV – TABLES, FIGURES, and REFERENCES

**Tables**

**Table 1. Experiment I Timeline**

| Date | Day of Study | | Measure/Activity |
|------|--------------|------|------------------|
| 17 Aug | Day 1 - **Phase 1** | | New Housing – FC & BW |
| 18Aug | Day 2 | | HCA |
| 19 Aug | Day 3 | | FC & OF |
| 20 Aug | Day 4 | | |
| 21 Aug | Day 5 | | FC & BW |
| 22 Aug | Day 6 | | |
| 23 Aug | Day 7 | | HCA & FC |
| 24 Aug | Day 8 | | |
| 25Aug | Day 9 | | FC |
| 26 Aug | Day 10 | | BW |
| 27 Aug | Day 11 | | FC |
| 28 Aug | Day 12 | | |
| 29 Aug | Day 13 | | FC & OF |
| 30 Aug | Day 14 | | HCA & BW |
| 31 Aug | Day 15 **- Phase 2** Day 1 | | FC |
| 1 Sep | Day 17 | Day 2 | HCA |
| 2 Sep | Day 18 | Day 3 | FC & OF |
| 3 Sep | Day 19 | Day 4 | |
| 4 Sep | Day 20 | Day 5 | FC & BW |
| 5 Sep | Day 21 | Day 6 | |
| 6 Sep | Day 22 | Day 7 | HCA |
| 7 Sep | Day 23 | Day 8 | |
| 8 Sep | Day 24 | Day 9 | FC |
| 9 Sep | Day 25 | Day 10 | BW |
| 10 Sep | Day 26 | Day 11 | FC |
| 11 Sep | Day 27 | Day 12 | |
| 12 Sep | Day 28 | Day 13 | FC & OF |
| 13 Sep | Day 29 | Day 14 | HCA & BW |
| 14 Sep | Day 30 **- Phase 3** Day 1 | | FC |
| 15 Sep | Day 31 | Day 2 | HCA |
| 16 Sep | Day 32 | Day 3 | FC & OF |
| 17 Sep | Day 33 | Day 4 | |
| 18 Sep | Day 34 | Day 5 | FC & BW |
| 19 Sep | Day 35 | Day 6 | |
| 20 Sep | Day 36 | Day 7 | HCA & FC |
| 21 Sep | Day 37 | Day 8 | |
| 22 Sep | Day 38 | Day 9 | FC |
| 23  Sep | Day 39 | Day 10 | BW |
| 24  Sep | Day 40 | Day 11 | FC |
| 25  Sep | Day 41 | Day 12 | |
| 26 Sep | Day 41 | Day 13 | FC & OF |
| 27 Sep | Day 43 | Day 14 | HCA & BW |
| 28 Sep | Day 44 | Day 15 | FC |
| 29Sep | Day 45 | Day 16 | HCA |
| 30 Sep | Day 46 | Day 17 | FC |
| 1 Oct | Day 47 | Day 18 | |
| 2 Oct | Day 48 | Day 19 | FC & BW |
| 3 Oct | Day 49 | Day 20 | HCA |
| 4 Oct | Day 50 | Day 21 | Sacrifice |

BW = Body Weight Measurement
FC = Food Consumption
HCA = Home Cage Activity
OF = Open Field (locomotor)

## Table 2. Experiment I Body Weight

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 13806.189 | 10 | 1380.619 | 8.185 | .000 | .204 | 1.000 |
| Time * Housing | 15522.627 | 20 | 776.131 | 4.601 | .000 | .223 | 1.000 |
| Error(time) | 53975.995 | 320 | 168.675 | | | | |
| **Tests of Between-Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 23472.350 | 2 | 11736.175 | 3.474 | .043 | .178 | .608 |
| Error | 108105.725 | 32 | 3378.304 | | | | |

| Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 95% Confidence Interval | |
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig.(a) | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 10.920 | 7.606 | .482 | -8.296 | 30.136 |
| | Super-Enriched | 19.204(*) | 7.287 | .039 | .793 | 37.616 |
| Enriched | Non-Enriched | -10.920 | 7.606 | .482 | -30.136 | 8.296 |
| | Super-Enriched | 8.284 | 7.254 | .786 | -10.042 | 26.611 |
| Super-Enriched | Non-Enriched | -19.204(*) | 7.287 | .039 | -37.616 | -.793 |
| | Enriched | -8.284 | 7.254 | .786 | -26.611 | 10.042 |

## Table 3. Experiment I Body Mass Index

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 529.719 | 2 | 264.859 | 6.682 | .004 | .288 | .888 |
| Error | 1308.032 | 33 | 39.637 | | | | |

| Post Hoc Comparisons - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 7.87103(*) | 2.570257 | .012 | 1.56415 | 14.17792 |
| | Super-Enriched | 8.37961(*) | 2.570257 | .007 | 2.07273 | 14.68649 |
| Enriched | Non-Enriched | -7.87103(*) | 2.570257 | .012 | -14.17792 | -1.56415 |
| | Super-Enriched | .50857 | 2.570257 | .979 | -5.79831 | 6.81546 |
| Super-Enriched | Non-Enriched | -8.37961(*) | 2.570257 | .007 | -14.68649 | -2.07273 |
| | Enriched | -.50857 | 2.570257 | .979 | -6.81546 | 5.79831 |

## Table 4. Experiment I Lee Index

### Tests of Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
|---|---|---|---|---|---|---|---|
| Housing | 1274.370 | 2 | 637.185 | 4.919 | .013 | .230 | .769 |
| Error | 4274.330 | 33 | 129.525 | | | | |

### Post Hoc Comparisons - Tukey HSD

| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 13.19140(*) | 4.646238 | .020 | 1.79049 | 24.59232 |
| | Super-Enriched | 11.96100(*) | 4.646238 | .038 | .56009 | 23.36191 |
| Enriched | Non-Enriched | -13.19140(*) | 4.646238 | .020 | -24.59232 | -1.79049 |
| | Super-Enriched | -1.23040 | 4.646238 | .962 | -12.63131 | 10.17051 |
| Super-Enriched | Non-Enriched | -11.96100(*) | 4.646238 | .038 | -23.36191 | -.56009 |
| | Enriched | 1.23040 | 4.646238 | .962 | -10.17051 | 12.63131 |

## Table 5a. Experiment I Phase A1 Gram Consumption

### Tests of Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
|---|---|---|---|---|---|---|---|
| Housing | 29.129 | 2 | 14.564 | 3.835 | .032 | .189 | .655 |
| Error | 125.335 | 33 | 3.798 | | | | |

### Post Hoc Comparisons - Tukey HSD

| (I) Housing | (J) housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | .007 | .7956 | 1.000 | -1.945 | 1.959 |
| | Super-Enriched | 1.912 | .7956 | .056 | -.041 | 3.864 |
| Enriched | Non-Enriched | -.007 | .7956 | 1.000 | -1.959 | 1.945 |
| | Super-Enriched | 1.905 | .7956 | .057 | -.048 | 3.857 |
| Super-Enriched | Non-Enriched | -1.912 | .7956 | .056 | -3.864 | .041 |
| | Enriched | -1.905 | .7956 | .057 | -3.857 | .048 |

## Table 5b. Experiment I Phase A1 Calorie Consumption

**Tests of Between-Subjects Effects**

| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
|---|---|---|---|---|---|---|---|
| Housing | 3.172 | 2 | 1.586 | 3.835 | .032 | .189 | .655 |
| Error | 13.649 | 33 | .414 | | | | |

**Post Hoc Comparisons - Tukey HSD**

| (I) housing | (J) housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | .002 | .2626 | 1.000 | -.642 | .647 |
| | Super-Enriched | .631 | .2626 | .056 | -.013 | 1.275 |
| Enriched | Non-Enriched | -.002 | .2626 | 1.000 | -.647 | .642 |
| | Super-Enriched | .629 | .2626 | .057 | -.016 | 1.273 |
| Super-Enriched | Non-Enriched | -.631 | .2626 | .056 | -1.275 | .013 |
| | Enriched | -.629 | .2626 | .057 | -1.273 | .016 |

## Table 5c. Experiment I Phase B Total Gram Consumption

**Tests of Between-Subjects Effects**

| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
|---|---|---|---|---|---|---|---|
| Housing | 7.400 | 2 | 3.700 | 1.151 | .329 | .065 | .235 |
| Error | 106.056 | 33 | 3.214 | | | | |

## Table 5d. Experiment I Phase B Total Calories Consumption

**Tests of Between-Subjects Effects**

| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
|---|---|---|---|---|---|---|---|
| Housing | 158.345 | 2 | 79.173 | 2.923 | .068 | .150 | .532 |
| Error | 893.873 | 33 | 27.087 | | | | |

## Table 5e. Experiment I Phase B Standard Chow Grams Consumption

**Tests of Between-Subjects Effects**

| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
|---|---|---|---|---|---|---|---|
| Housing | 23.053 | 2 | 11.527 | 3.767 | .034 | .186 | .647 |
| Error | 100.990 | 33 | 3.060 | | | | |

**Post Hoc Comparisons - Tukey HSD**

| (I) housing | (J) housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | -1.5447 | .71418 | .093 | -3.2971 | .2077 |
| | Super-Enriched | -1.8174(*) | .71418 | .041 | -3.5698 | -.0649 |
| Enriched | Non-Enriched | 1.5447 | .71418 | .093 | -.2077 | 3.2971 |
| | Super-Enriched | -.2727 | .71418 | .923 | -2.0251 | 1.4798 |
| Super-Enriched | Non-Enriched | 1.8174(*) | .71418 | .041 | .0649 | 3.5698 |
| | Enriched | .2727 | .71418 | .923 | -1.4798 | 2.0251 |

## Table 5f. Experiment I Phase B Standard Chow Calories Consumption

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 10.042 | 2 | 5.021 | 3.767 | .034 | .186 | .647 |
| Error | 43.991 | 33 | 1.333 | | | | |

| Post Hoc Comparisons - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) housing | (J) housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | -1.5447 | .71418 | .093 | -3.2971 | .2077 |
| | Super-Enriched | -1.8174(*) | .71418 | .041 | -3.5698 | -.0649 |
| Enriched | Non-Enriched | 1.5447 | .71418 | .093 | -.2077 | 3.2971 |
| | Super-Enriched | -.2727 | .71418 | .923 | -2.0251 | 1.4798 |
| Super-Enriched | Non-Enriched | 1.8174(*) | .71418 | .041 | .0649 | 3.5698 |
| | Enriched | .2727 | .71418 | .923 | -1.4798 | 2.0251 |

## Table 5g. Experiment I Phase B Oreo Cookie Grams Consumption

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 1.051 | 2 | .526 | .343 | .712 | .020 | .100 |
| Error | 50.637 | 33 | 1.534 | | | | |

## Table 5h. Experiment I Phase B Oreo Cookie Calories

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 23.319 | 2 | 11.660 | .343 | .712 | .020 | .100 |
| Error | 1123.335 | 33 | 34.040 | | | | |

## Table 5i. Experiment I Phase B Lay's Chip Gram Consumption

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 3.831 | 2 | 1.915 | 2.078 | .141 | .112 | .397 |
| Error | 30.418 | 33 | .922 | | | | |

## Table 5j. Experiment I Phase B Lay's Chip Calorie Consumption

| Tests of Between-Subjects Effects | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 110.061 | 2 | 55.030 | 2.078 | .141 | .112 | .397 |
| Error | 873.909 | 33 | 26.482 | | | | |

## Table 5k. Experiment I Phase A2 Standard Chow Gram Consumption

| Tests of Between-Subjects Effects | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 65.730 | 2 | 32.865 | 1.654 | .207 | .091 | .323 |
| Error | 125.324 | 33 | 3.798 | | | | |

## Table 5l. Experiment I Phase A2 Standard Chow Calorie Consumption

| Tests of Between-Subjects Effects | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 5.528 | 2 | 2.764 | 1.532 | .231 | .085 | .302 |
| Error | 59.552 | 33 | 1.805 | | | | |

## Table 6a. Experiment I Phase B Home Cage Activity

| Kruskal-Wallis Test | Housing Condition | N | Mean Rank |
| --- | --- | --- | --- |
| **Number of Animals Moving** | Non-Enriched | 6 | 6.08 |
| | Enriched | 6 | 9.75 |
| | Super-Enriched | 6 | 12.67 |
| | Total | 18 | |
| **Amount of Activity** | Non-Enriched | 6 | 5.00 |
| | Enriched | 6 | 10.08 |
| | Super-Enriched | 6 | 13.42 |
| | Total | 18 | |
| **Level of Activity** | Non-Enriched | 6 | 4.67 |
| | Enriched | 6 | 11.92 |
| | Super-Enriched | 6 | 11.92 |
| | Total | 18 | |

| Grouping Variable: Housing Condition | Number Moving | Activity Amount | Activity Level |
| --- | --- | --- | --- |
| Chi-Square | 4.769 | 8.493 | 7.517 |
| Df | 2 | 2 | 2 |
| Asymp. Sig. | .092 | .014 | .023 |

## Table 6b. Experiment I Phase A2 Home Cage Activity

| Kruskal-Wallis Test | Housing Condition | N | Mean Rank |
|---|---|---|---|
| **Number of Animals Moving** | Non-Enriched | 11 | 10.59 |
| | Enriched | 11 | 21.45 |
| | Super-Enriched | 11 | 18.95 |
| | Total | 33 | |
| **Amount of Activity** | Non-Enriched | 11 | 8.50 |
| | Enriched | 11 | 20.91 |
| | Super-Enriched | 11 | 21.59 |
| | Total | 33 | |
| **Level of Activity** | Non-Enriched | 11 | 7.82 |
| | Enriched | 11 | 20.00 |
| | Super-Enriched | 11 | 23.18 |
| | Total | 33 | |
| **Grouping Variable: Housing Condition** | **Animals Moving** | **Amount Activity** | **Level Activity** |
| Chi-Square | 10.293 | 13.233 | 16.106 |
| Df | 2 | 2 | 2 |
| Asymp. Sig. | .006 | .001 | .000 |

## Table 7a. Experiment I Open Field MANOVA

| Dependent Variable | | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
|---|---|---|---|---|---|---|---|---|
| TOTHAC1 | Contrast | 100342713.722 | 2 | 50171356.861 | 7.716 | .002 | .319 | .929 |
| | Error | 214583221.917 | 33 | 6502521.876 | | | | |
| TOTVAC1 | Contrast | 276447.167 | 2 | 138223.583 | 3.362 | .047 | .169 | .594 |
| | Error | 1356918.833 | 33 | 41118.753 | | | | |
| TOTHAC2 | Contrast | 471468284.056 | 2 | 235734142.028 | 20.947 | .000 | .559 | 1.000 |
| | Error | 371374981.833 | 33 | 11253787.328 | | | | |
| TOTVAC2 | Contrast | 1332567.167 | 2 | 666283.583 | 14.120 | .000 | .461 | .997 |
| | Error | 1557191.833 | 33 | 47187.631 | | | | |
| TOTHAC3 | Contrast | 289371641.167 | 2 | 144685820.583 | 13.394 | .000 | .448 | .996 |
| | Error | 356477343.833 | 33 | 10802343.753 | | | | |
| TOTVAC3 | Contrast | 643658.167 | 2 | 321829.083 | 8.966 | .001 | .352 | .960 |
| | Error | 1184473.833 | 33 | 35893.146 | | | | |
| TOTHAC4 | Contrast | 319840198.389 | 2 | 159920099.194 | 9.464 | .001 | .365 | .969 |
| | Error | 557616582.833 | 33 | 16897472.207 | | | | |
| TOTVAC4 | Contrast | 1380348.667 | 2 | 690174.333 | 6.198 | .005 | .273 | .862 |
| | Error | 3674640.083 | 33 | 111352.730 | | | | |
| TOTHAC5 | Contrast | 404176753.500 | 2 | 202088376.750 | 24.785 | .000 | .600 | 1.000 |
| | Error | 269072695.250 | 33 | 8153718.038 | | | | |
| TOTVAC5 | Contrast | 2599014.500 | 2 | 1299507.250 | 13.373 | .000 | .448 | .996 |
| | Error | 3206798.250 | 33 | 97175.705 | | | | |
| TOTHAC6 | Contrast | 632487582.167 | 2 | 316243791.083 | 16.079 | .000 | .494 | .999 |
| | Error | 649038160.833 | 33 | 19667823.056 | | | | |
| TOTVAC6 | Contrast | 8956486.167 | 2 | 4478243.083 | 11.735 | .000 | .416 | .990 |
| | Error | 12593082.583 | 33 | 381608.563 | | | | |

## Table 7b. Experiment I OF 1 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 49633230.414 | 11 | 4512111.856 | 30.459 | .000 | .480 | 1.000 |
| TIME *HOUSING | 12470439.079 | 22 | 566838.140 | 3.826 | .000 | .188 | 1.000 |
| Error(TIME) | 53773780.924 | 363 | 148137.138 | | | | |
| **Tests of Between-Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 8361892.810 | 2 | 4180946.405 | 7.716 | .002 | .319 | .929 |
| Error | 17881935.160 | 33 | 541876.823 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 83.042 | 86.753 | 1.000 | -135.768 | 301.851 |
| | Super-Enriched | 327.757(*) | 86.753 | .002 | 108.948 | 546.566 |
| Enriched | Non-Enriched | -83.042 | 86.753 | 1.000 | -301.851 | 135.768 |
| | Super-Enriched | 244.715(*) | 86.753 | .024 | 25.906 | 463.525 |
| Super-Enriched | Non-Enriched | -327.757(*) | 86.753 | .002 | -546.566 | -108.948 |
| | Enriched | -244.715(*) | 86.753 | .024 | -463.525 | -25.906 |

## Table 7c. Experiment I OF1 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 176856.944 | 11 | 16077.904 | 14.704 | .000 | .308 | 1.000 |
| TIME * HOUSING | 60230.292 | 22 | 2737.741 | 2.504 | .000 | .132 | .999 |
| Error(TIME) | 396919.597 | 363 | 1093.442 | | | | |
| **Tests of Between-Subjects Effects** | | | | | | | |
| Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 23037.264 | 2 | 11518.632 | 3.362 | .047 | .169 | .594 |
| Error | 113076.569 | 33 | 3426.563 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | .243 | 6.899 | 1.000 | -17.157 | 17.643 |
| | Super-Enriched | 15.611 | 6.899 | .091 | -1.789 | 33.011 |
| Enriched | Non-Enriched | -.243 | 6.899 | 1.000 | -17.643 | 17.157 |
| | Super-Enriched | 15.368 | 6.899 | .099 | -2.032 | 32.768 |
| Super-Enriched | Non-Enriched | -15.611 | 6.899 | .091 | -33.011 | 1.789 |
| | Enriched | -15.368 | 6.899 | .099 | -32.768 | 2.032 |

## Table 7d. Experiment I OF 2 Horizontal Activity

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Tests of Within-Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 105817108.935 | 11 | 9619737.176 | 47.499 | .000 | .590 | 1.000 |
| TIME*HOUSING | 28284114.384 | 22 | 1285641.563 | 6.348 | .000 | .278 | 1.000 |
| Error(TIME) | 73515888.514 | 363 | 202523.109 | | | | |
| **Tests of Between-Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 39289023.671 | 2 | 19644511.836 | 20.947 | .000 | .559 | 1.000 |
| Error | 30947915.153 | 33 | 937815.611 | | | | |

| **Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 130.563 | 114.128 | .783 | -157.293 | 418.418 |
| | Super-Enriched | 694.944(*) | 114.128 | .000 | 407.089 | 982.800 |
| Enriched | Non-Enriched | -130.563 | 114.128 | .783 | -418.418 | 157.293 |
| | Super-Enriched | 564.382(*) | 114.128 | .000 | 276.527 | 852.237 |
| Super-Enriched | Non-Enriched | -694.944(*) | 114.128 | .000 | -982.800 | -407.089 |
| | Enriched | -564.382(*) | 114.128 | .000 | -852.237 | -276.527 |

## Table 7e. Experiment I OF 2 Vertical Activity

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Tests of Within-Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 595294.750 | 11 | 54117.705 | 39.802 | .000 | .547 | 1.000 |
| TIME*HOUSING | 150607.569 | 22 | 6845.799 | 5.035 | .000 | .234 | 1.000 |
| Error(TIME) | 493566.347 | 363 | 1359.687 | | | | |
| **Tests of Between-Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 111047.264 | 2 | 55523.632 | 14.120 | .000 | .461 | .997 |
| Error | 129765.986 | 33 | 3932.303 | | | | |

| **Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | -6.056 | 7.390 | 1.000 | -24.695 | 12.584 |
| | Super-Enriched | 30.576(*) | 7.390 | .001 | 11.937 | 49.216 |
| Enriched | Non-Enriched | 6.056 | 7.390 | 1.000 | -12.584 | 24.695 |
| | Super-Enriched | 36.632(*) | 7.390 | .000 | 17.992 | 55.272 |
| Super-Enriched | Non-Enriched | -30.576(*) | 7.390 | .001 | -49.216 | -11.937 |
| | Enriched | -36.632(*) | 7.390 | .000 | -55.272 | -17.992 |

## Table 7f. Experiment I OF 3 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 115655171.583 | 11 | 10514106.508 | 47.521 | .000 | .590 | 1.000 |
| TIME*HOUSING | 21794337.069 | 22 | 990651.685 | 4.477 | .000 | .213 | 1.000 |
| Error(TIME) | 80314194.514 | 363 | 221251.225 | | | | |
| Tests of Between-Subjects Effects | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 24114303.431 | 2 | 12057151.715 | 13.394 | .000 | .448 | .996 |
| Error | 29706445.319 | 33 | 900195.313 | | | | |

| Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 1.528 | 111.816 | 1.000 | -280.495 | 283.550 |
| | Super-Enriched | 501.951(*) | 111.816 | .000 | 219.929 | 783.974 |
| Enriched | Non-Enriched | -1.528 | 111.816 | 1.000 | -283.550 | 280.495 |
| | Super-Enriched | 500.424(*) | 111.816 | .000 | 218.401 | 782.446 |
| Super-Enriched | Non-Enriched | -501.951(*) | 111.816 | .000 | -783.974 | -219.929 |
| | Enriched | -500.424(*) | 111.816 | .000 | -782.446 | -218.401 |

## Table 7g. Experiment I OF3 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 576140.056 | 11 | 52376.369 | 40.548 | .000 | .551 | 1.000 |
| TIME*HOUSING | 68415.764 | 22 | 3109.807 | 2.408 | .000 | .127 | .998 |
| Error(TIME) | 468887.514 | 363 | 1291.701 | | | | |
| Tests of Between-Subjects Effects | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 53638.181 | 2 | 26819.090 | 8.966 | .001 | .352 | .960 |
| Error | 98706.153 | 33 | 2991.096 | | | | |

| Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 1.660 | 6.445 | 1.000 | -14.597 | 17.916 |
| | Super-Enriched | 24.424(*) | 6.445 | .002 | 8.167 | 40.680 |
| Enriched | Non-Enriched | -1.660 | 6.445 | 1.000 | -17.916 | 14.597 |
| | Super-Enriched | 22.764(*) | 6.445 | .004 | 6.507 | 39.021 |
| Super-Enriched | Non-Enriched | -24.424(*) | 6.445 | .002 | -40.680 | -8.167 |
| | Enriched | -22.764(*) | 6.445 | .004 | -39.021 | -6.507 |

## Table 7h. Experiment I OF 4 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 194173584.444 | 11 | 17652144.040 | 85.290 | .000 | .721 | 1.000 |
| TIME*HOUSING | 20375592.681 | 22 | 926163.304 | 4.475 | .000 | .213 | 1.000 |
| Error(TIME) | 75128572.542 | 363 | 206965.765 | | | | |
| **Tests of Between-Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 26335164.542 | 2 | 13167582.271 | 9.604 | .001 | .368 | .971 |
| Error | 45243536.458 | 33 | 1371016.256 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 381.562(*) | 137.992 | .028 | 33.516 | 729.609 |
| | Super-Enriched | 597.146(*) | 137.992 | .000 | 249.100 | 945.192 |
| Enriched | Non-Enriched | -381.562(*) | 137.992 | .028 | -729.609 | -33.516 |
| | Super-Enriched | 215.583 | 137.992 | .383 | -132.463 | 563.629 |
| Super-Enriched | Non-Enriched | -597.146(*) | 137.992 | .000 | -945.192 | -249.100 |
| | Enriched | -215.583 | 137.992 | .383 | -563.629 | 132.463 |

## Table 7i. Experiment I OF 4 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 1309952.303 | 11 | 119086.573 | 53.655 | .000 | .619 | 1.000 |
| TIME*HOUSING | 131486.981 | 22 | 5976.681 | 2.693 | .000 | .140 | .999 |
| Error(TIME) | 805679.299 | 363 | 2219.502 | | | | |
| **Tests of Between-Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 112342.019 | 2 | 56171.009 | 6.186 | .005 | .273 | .862 |
| Error | 299639.451 | 33 | 9079.983 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 28.375(*) | 11.230 | .049 | .051 | 56.699 |
| | Super-Enriched | 37.986(*) | 11.230 | .006 | 9.662 | 66.310 |
| Enriched | Non-Enriched | -28.375(*) | 11.230 | .049 | -56.699 | -.051 |
| | Super-Enriched | 9.611 | 11.230 | 1.000 | -18.713 | 37.935 |
| Super-Enriched | Non-Enriched | -37.986(*) | 11.230 | .006 | -66.310 | -9.662 |
| | Enriched | -9.611 | 11.230 | 1.000 | -37.935 | 18.713 |

## Table 7j. Experiment I OF 5 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 181861445.674 | 11 | 16532858.698 | 80.477 | .000 | .709 | 1.000 |
| TIME*HOUSING | 13818534.431 | 22 | 628115.201 | 3.057 | .000 | .156 | 1.000 |
| Error(TIME) | 74573405.479 | 363 | 205436.379 | | | | |

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 33681396.125 | 2 | 16840698.063 | 24.785 | .000 | .600 | 1.000 |
| Error | 22422724.604 | 33 | 679476.503 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 275.146(*) | 97.145 | .023 | 30.125 | 520.167 |
| | Super-Enriched | 679.854(*) | 97.145 | .000 | 434.833 | 924.875 |
| Enriched | Non-Enriched | -275.146(*) | 97.145 | .023 | -520.167 | -30.125 |
| | Super-Enriched | 404.708(*) | 97.145 | .001 | 159.688 | 649.729 |
| Super-Enriched | Non-Enriched | -679.854(*) | 97.145 | .000 | -924.875 | -434.833 |
| | Enriched | -404.708(*) | 97.145 | .001 | -649.729 | -159.688 |

## Table 7k. Experiment I OF 5 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 1509106.562 | 11 | 137191.506 | 61.300 | .000 | .650 | 1.000 |
| TIME*HOUSING | 136050.625 | 22 | 6184.119 | 2.763 | .000 | .143 | 1.000 |
| Error(TIME) | 812405.896 | 363 | 2238.033 | | | | |

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 216584.542 | 2 | 108292.271 | 13.373 | .000 | .448 | .996 |
| Error | 267233.188 | 33 | 8097.975 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 22.646 | 10.605 | .121 | -4.103 | 49.395 |
| | Super-Enriched | 54.583(*) | 10.605 | .000 | 27.835 | 81.332 |
| Enriched | Non-Enriched | -22.646 | 10.605 | .121 | -49.395 | 4.103 |
| | Super-Enriched | 31.938(*) | 10.605 | .015 | 5.189 | 58.686 |
| Super-Enriched | Non-Enriched | -54.583(*) | 10.605 | .000 | -81.332 | -27.835 |
| | Enriched | -31.938(*) | 10.605 | .015 | -58.686 | -5.189 |

## Table 7l. Experiment I OF 6 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 178523926.639 | 11 | 16229447.876 | 72.093 | .000 | .686 | 1.000 |
| TIME*HOUSING | 19018308.764 | 22 | 864468.580 | 3.840 | .000 | .189 | 1.000 |
| Error(TIME) | 81718428.597 | 363 | 225119.638 | | | | |

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 52707298.514 | 2 | 26353649.257 | 16.079 | .000 | .494 | .999 |
| Error | 54086513.403 | 33 | 1638985.255 | | | | |

| Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference(a) | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 261.132 | 150.876 | .279 | -119.410 | 641.674 |
| | Super-Enriched | 836.181(*) | 150.876 | .000 | 455.638 | 1216.723 |
| Enriched | Non-Enriched | -261.132 | 150.876 | .279 | -641.674 | 119.410 |
| | Super-Enriched | 575.049(*) | 150.876 | .002 | 194.506 | 955.591 |
| Super-Enriched | Non-Enriched | -836.181(*) | 150.876 | .000 | -1216.723 | -455.638 |
| | Enriched | -575.049(*) | 150.876 | .002 | -955.591 | -194.506 |

## Table 7m. Experiment I OF 6 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| TIME | 2005099.618 | 11 | 182281.783 | 41.351 | .000 | .556 | 1.000 |
| TIME*HOUSING | 216221.597 | 22 | 9828.254 | 2.230 | .001 | .119 | .996 |
| Error(TIME) | 1600161.368 | 363 | 4408.158 | | | | |

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| HOUSING | 746373.847 | 2 | 373186.924 | 11.735 | .000 | .416 | .990 |
| Error | 1049423.549 | 33 | 31800.714 | | | | |

| Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) HOUSING | (J) HOUSING | Mean Difference (I-J) | Std. Error | Sig.(a) | 95% Confidence Interval for Difference(a) | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 36.660 | 21.016 | .271 | -16.347 | 89.667 |
| | Super-Enriched | 100.590(*) | 21.016 | .000 | 47.583 | 153.597 |
| Enriched | Non-Enriched | -36.660 | 21.016 | .271 | -89.667 | 16.347 |
| | Super-Enriched | 63.931(*) | 21.016 | .014 | 10.923 | 116.938 |
| Super-Enriched | Non-Enriched | -100.590(*) | 21.016 | .000 | -153.597 | -47.583 |
| | Enriched | -63.931(*) | 21.016 | .014 | -116.938 | -10.923 |

**Table 8. Experiment II Timeline**

| Date | Day of Study | | Measure/Activity |
|---|---|---|---|
| 25 Jan | Day 1 - **Phase 1** | | New Housing – FC & BW |
| 26 Jan | Day 2 | | HCA |
| 27 Jan | Day 3 | | FC & OF |
| 28 Jan | Day 4 | | |
| 29 Jan | Day 5 | | FC & BW |
| 30 Jan | Day 6 | | |
| 31 Jan | Day 7 | | HCA & FC |
| 1 Feb | Day 8 | | |
| 2 Feb | Day 9 | | FC |
| 3 Feb | Day 10 | | BW |
| 4 Feb | Day 11 | | FC |
| 5 Feb | Day 12 | | |
| 6 Feb | Day 13 | | FC |
| 7 Feb | Day 14 | | HCA & BW& OF |
| 8 Feb | Day 15 **- Phase 2** | Day 1 | FC |
| 9 Feb | Day 17 | Day 2 | HCA |
| 10 Feb | Day 18 | Day 3 | FC & OF |
| 11 Feb | Day 19 | Day 4 | |
| 12 Feb | Day 20 | Day 5 | FC & BW |
| 13 Feb | Day 21 | Day 6 | |
| 14 Feb | Day 22 | Day 7 | HCA |
| 15 Feb | Day 23 | Day 8 | |
| 16 Feb | Day 24 | Day 9 | FC |
| 17 Feb | Day 25 | Day 10 | BW |
| 18 Feb | Day 26 | Day 11 | FC |
| 19 Feb | Day 27 | Day 12 | |
| 20 Feb | Day 28 | Day 13 | FC |
| 21 Feb | Day 29 | Day 14 | HCA & BW& OF |
| 22 Feb | Day 30 **- Phase 3** | Day 1 | FC |
| 23 Feb | Day 31 | Day 2 | HCA |
| 24 Feb | Day 32 | Day 3 | FC & OF |
| 25 Feb | Day 33 | Day 4 | |
| 26 Feb | Day 34 | Day 5 | FC & BW |
| 27 Feb | Day 35 | Day 6 | |
| 28 Feb | Day 36 | Day 7 | HCA & FC |
| 29 Feb | Day 37 | Day 8 | |
| 30 Feb | Day 38 | Day 9 | FC |
| 31 Feb | Day 39 | Day 10 | BW |
| 1 Mar | Day 40 | Day 11 | FC |
| 2 Mar | Day 41 | Day 12 | |
| 3 Mar | Day 41 | Day 13 | FC |
| 4 Mar | Day 43 | Day 14 | HCA & BW |
| 5 Mar | Day 44 | Day 15 | FC |
| 6 Mar | Day 45 | Day 16 | HCA |
| 7 Mar | Day 46 | Day 17 | FC |
| 8 Mar | Day 47 | Day 18 | |
| 9 Mar | Day 48 | Day 19 | FC & BW |
| 10 Mar | Day 49 | Day 20 | HCA & OF |
| 11 Mar | Day 50 | Day 21 | Sacrifice |

BW = Body Weight Measurement
FC = Food Consumption
HCA = Home Cage Activity
OF = Open Field (locomotor)

## Table 9. Experiment II Body Weight

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 9486.911 | 10 | 948.691 | 12.554 | .000 | .282 | 1.000 |
| Time * Housing | 4658.486 | 20 | 232.924 | 3.082 | .000 | .162 | 1.000 |
| Error(time) | 24182.357 | 320 | 75.570 | | | | |
| Tests of Between -Subjects Effects | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| BW Base | 21314.159 | 1 | 21314.159 | 12.380 | .001 | .279 | .927 |
| Housing | 26951.043 | 2 | 13475.522 | 7.827 | .002 | .328 | .932 |
| Error | 55094.273 | 32 | 1721.696 | | | | |
| Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | | |
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | | |
| | | | | | Lower Bound | Upper Bound | |
| Non-Enriched | Enriched | 4.687 | 5.130 | 1.000 | -8.274 | 17.649 | |
| | Super-Enriched | 19.440(*) | 5.115 | .002 | 6.518 | 32.362 | |
| Enriched | Non-Enriched | -4.687 | 5.130 | 1.000 | -17.649 | 8.274 | |
| | Super-Enriched | 14.753(*) | 5.163 | .022 | 1.709 | 27.797 | |
| Super-Enriched | Non-Enriched | -19.440(*) | 5.115 | .002 | -32.362 | -6.518 | |
| | Enriched | -14.753(*) | 5.163 | .022 | -27.797 | -1.709 | |

## Table 10. Experiment II Body Mass Index

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 263.824 | 2 | 131.912 | 6.836 | .003 | .293 | .895 |
| Error | 636.769 | 33 | 19.296 | | | | |
| Post Hoc Comparisons - Tukey HSD | | | | | | | |
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | | |
| | | | | | Lower Bound | Upper Bound | |
| Non-Enriched | Enriched | -.148 | 1.793 | 1.000 | -4.671 | 4.375 | |
| | Super-Enriched | 5.667(*) | 1.793 | .010 | 1.144 | 10.190 | |
| Enriched | Non-Enriched | .148 | 1.793 | 1.000 | -4.375 | 4.671 | |
| | Super-Enriched | 5.815(*) | 1.793 | .008 | 1.292 | 10.338 | |
| Super-Enriched | Non-Enriched | -5.667(*) | 1.793 | .010 | -10.190 | -1.144 | |
| | Enriched | -5.815(*) | 1.793 | .008 | -10.338 | -1.292 | |

## Table 11. Experiment II Lee Index

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 782.285 | 2 | 391.142 | 4.342 | .021 | .208 | .713 |
| Error | 2972.896 | 33 | 90.088 | | | | |
| **Post Hoc Comparisons - Tukey HSD** | | | | | | | |
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | | |
| | | | | | Lower Bound | Upper Bound | |
| Non-Enriched | Enriched | -2.529 | 3.875 | 1.000 | -12.302 | 7.244 | |
| | Super-Enriched | 8.379 | 3.875 | .114 | -1.395 | 18.152 | |
| Enriched | Non-Enriched | 2.529 | 3.875 | 1.000 | -7.244 | 12.302 | |
| | Super-Enriched | 10.908(*) | 3.875 | .024 | 1.134 | 20.681 | |
| Super-Enriched | Non-Enriched | -8.379 | 3.875 | .114 | -18.152 | 1.395 | |
| | Enriched | -10.908(*) | 3.875 | .024 | -20.681 | -1.134 | |

## Table 12a. Experiment II Phase A1 Gram Consumption

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 26.820 | 2 | 13.410 | 2.526 | .095 | .133 | .470 |
| Error | 175.161 | 33 | 5.308 | | | | |

## Table 12b. Experiment II Phase A1 Calorie Consumption

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power(a) |
| Housing | 9.890 | 2 | 4.945 | 18.766 | .000 | .532 | 1.000 |
| Error | 8.696 | 33 | .264 | | | | |
| **Tests of Between-Subjects Effects Post Hoc Comparisons** - Tukey HSD | | | | | | | |
| (I) housing | (J) housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | | |
| | | | | | Lower Bound | Upper Bound | |
| Non-Enriched | Enriched | 1.237 (*) | .2095671 | .000 | .723030 | 1.751499 | |
| | Super-Enriched | .9155 (*) | .2095671 | .000 | .401280 | 1.429749 | |
| Enriched | Non-Enriched | -1.237 (*) | .2095671 | .000 | -1.751499 | -.723030 | |
| | Super-Enriched | -.3217 | .2095671 | .288 | -.835985 | .192485 | |
| Super-Enriched | Non-Enriched | -.9155 (*) | .2095671 | .000 | -1.429749 | -.401280 | |
| | Enriched | .3217 | .2095671 | .288 | -.192485 | .835985 | |

## Table 12c. Experiment II Phase B Total Gram Consumption

| | | | Tests of Between-Subjects Effects | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 69.627 | 2 | 34.814 | 6.017 | .006 | .267 | .852 |
| Error | 190.932 | 33 | 5.786 | | | | |

| | | Post Hoc Comparisons - Tukey HSD | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 1.273333 | .9819893 | .407 | -1.136267 | 3.682933 |
| | Super-Enriched | 3.372976(*) | .9819893 | .004 | .963376 | 5.782576 |
| Enriched | Non-Enriched | -1.273333 | .9819893 | .407 | -3.682933 | 1.136267 |
| | Super-Enriched | 2.099643 | .9819893 | .098 | -.309957 | 4.509243 |
| Super-Enriched | Non-Enriched | -3.372976(*) | .9819893 | .004 | -5.782576 | -.963376 |
| | Enriched | -2.099643 | .9819893 | .098 | -4.509243 | .309957 |

## Table 12d. Experiment II Phase B Total Calories Consumption

| | | | Tests of Between-Subjects Effects | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 2323.958 | 2 | 1161.979 | 12.753 | .000 | .436 | .994 |
| Error | 3006.718 | 33 | 91.113 | | | | |

| | | Post Hoc Comparisons - Tukey HSD | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 10.709573(*) | 3.8968507 | .025 | 1.147502 | 20.271643 |
| | Super-Enriched | 19.654212(*) | 3.8968507 | .000 | 10.092141 | 29.216283 |
| Enriched | Non-Enriched | -10.709573(*) | 3.8968507 | .025 | -20.271643 | -1.147502 |
| | Super-Enriched | 8.944639 | 3.8968507 | .070 | -.617432 | 18.506710 |
| Super-Enriched | Non-Enriched | -19.654212(*) | 3.8968507 | .000 | -29.216283 | -10.092141 |
| | Enriched | -8.944639 | 3.8968507 | .070 | -18.506710 | .617432 |

## Table 12e. Experiment II Phase B Standard Chow Grams Consumption

| | | | Tests of Between-Subjects Effects | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 26.820 | 2 | 13.410 | 2.526 | .095 | .133 | .470 |
| Error | 175.161 | 33 | 5.308 | | | | |

### Table 12f. Experiment II Phase B Standard Chow Calories Consumption

| Tests of Between-Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 3.479 | 2 | 1.739 | 2.526 | .095 | .133 | .470 |
| Error | 22.722 | 33 | .689 | | | | |

### Table 12g. Experiment II Phase B Oreo Cookie Grams Consumption

| Tests of Between-Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 2007.224 | 2 | 1003.612 | 13.889 | .000 | .457 | .997 |
| Error | 2384.527 | 33 | 72.258 | | | | |

| Post Hoc Comparisons -Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 8.9804(*) | 3.470 | .037 | .465 | 17.495 |
| | Super-Enriched | 18.2894(*) | 3.470 | .000 | 9.773 | 26.804 |
| Enriched | Non-Enriched | -8.9804(*) | 3.470 | .037 | -17.495 | -.465 |
| | Super-Enriched | 9.3090(*) | 3.470 | .030 | .793 | 17.824 |
| Super-Enriched | Non-Enriched | -18.2894(*) | 3.470 | .000 | -26.804 | -9.773 |
| | Enriched | -9.3090(*) | 3.470 | .030 | -17.824 | -.793 |

### Table 12h. Experiment II Phase B Oreo Cookie Calories Consumption

| Tests of Between-Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 2007.224 | 2 | 1003.612 | 13.889 | .000 | .457 | .997 |
| Error | 2384.527 | 33 | 72.258 | | | | |

| Post Hoc Comparisons - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 8.980 (*) | 3.470 | .037 | .464 | 17.495 |
| | Super-Enriched | 18.289 (*) | 3.470 | .000 | 9.773 | 26.804 |
| Enriched | Non-Enriched | -8.980 (*) | 3.470 | .037 | -17.495 | -.464 |
| | Super-Enriched | 9.308 (*) | 3.470 | .030 | .793 | 17.824 |
| Super-Enriched | Non-Enriched | -18.289 (*) | 3.470 | .000 | -26.804 | -9.773 |
| | Enriched | -9.308 (*) | 3.470 | .030 | -17.824 | -.793 |

### Table 12i. Experiment II Phase B Lay's Chip Grams Consumption

| Tests of Between-Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 5.580 | 2 | 2.790 | .666 | .521 | .039 | .152 |
| Error | 138.340 | 33 | 4.192 | | | | |

## Table 12j. Experiment II Phase B Lay's Chip Calorie Consumption

| | | | | | Tests of Between-Subjects Effects | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 40.079 | 2 | 20.039 | .666 | .521 | .039 | .152 |
| Error | 993.610 | 33 | 30.109 | | | | |

## Table 12k. Experiment II Phase A2 Standard Chow Gram Consumption

| | | | | | Tests of Between-Subjects Effects | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 83.267 | 2 | 41.633 | 8.749 | .001 | .347 | .956 |
| Error | 157.026 | 33 | 4.758 | | | | |

| Post Hoc Comparisons - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 2.019832 | .890 | .075 | -.165370 | 4.205034 |
| | Super-Enriched | -1.700901 | .890 | .152 | -3.886103 | .484301 |
| Enriched | Non-Enriched | -2.019832 | .890 | .075 | -4.205034 | .165370 |
| | Super-Enriched | -3.720733(*) | .890 | .001 | -5.905935 | -1.535531 |
| Super-Enriched | Non-Enriched | 1.700901 | .890 | .152 | -.484301 | 3.886103 |
| | Enriched | 3.720733(*) | .890 | .001 | 1.535531 | 5.905935 |

## Table 12l. Experiment I Phase A2 Standard Chow Calorie Consumption

| | | | | | Tests of Between-Subjects Effects | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 2323.958 | 2 | 1161.979 | 12.753 | .000 | .436 | .994 |
| Error | 3006.718 | 33 | 91.113 | | | | |

| Post Hoc Comparisons - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 10.709(*) | 3.896 | .025 | 1.147502 | 20.271643 |
| | Super-Enriched | 19.654(*) | 3.896 | .000 | 10.092141 | 29.216283 |
| Enriched | Non-Enriched | -10.709(*) | 3.896 | .025 | -20.271643 | -1.147502 |
| | Super-Enriched | 8.944 | 3.896 | .070 | -.617432 | 18.506710 |
| Super-Enriched | Non-Enriched | -19.654(*) | 3.896 | .000 | -29.216283 | -10.092141 |
| | Enriched | -8.944 | 3.896 | .070 | -18.506710 | .617432 |

**Table 13a. Experiment II Phase A1 Home Cage Activity Kruskal Wallis Test**

| Kruskal-Wallis Test | Housing Condition | N | Mean Rank |
|---|---|---|---|
| **Number of Animals Moving** | Non-Enriched | 10 | 13.10 |
| | Enriched | 10 | 14.70 |
| | Super-Enriched | 10 | 18.70 |
| | Total | 30 | |
| **Amount of Activity** | Non-Enriched | 10 | 10.70 |
| | Enriched | 10 | 14.75 |
| | Super-Enriched | 10 | 21.05 |
| | Total | 30 | |
| **Level of Activity** | Non-Enriched | 10 | 10.50 |
| | Enriched | 10 | 13.90 |
| | Super-Enriched | 10 | 22.10 |
| | Total | 30 | |

| Grouping Variable: Housing Condition | Number Moving | Activity Amount | Activity Level |
|---|---|---|---|
| Chi-Square | 2.872 | 7.381 | 9.683 |
| Df | 2 | 2 | 2 |
| Asymp. Sig. | .238 | .025 | .008 |

**Table 13b. Experiment II Phase B Home Cage Activity Kruskal Wallis Test**

| Kruskal-Wallis Test | Housing Condition | N | Mean Rank |
|---|---|---|---|
| **Number of Animals Moving** | Non-Enriched | 10 | 15.50 |
| | Enriched | 10 | 15.50 |
| | Super-Enriched | 10 | 15.50 |
| | Total | 30 | |
| **Amount of Activity** | Non-Enriched | 10 | 6.90 |
| | Enriched | 10 | 18.40 |
| | Super-Enriched | 10 | 21.20 |
| | Total | 30 | |
| **Level of Activity** | Non-Enriched | 10 | 6.00 |
| | Enriched | 10 | 17.00 |
| | Super-Enriched | 10 | 23.50 |
| | Total | 30 | |

| Grouping Variable: Housing Condition | Number Moving | Activity Amount | Activity Level |
|---|---|---|---|
| Chi-Square | .000 | 16.130 | 21.909 |
| Df | 2 | 2 | 2 |
| Asymp. Sig. | 1.000 | .000 | .000 |

## Table 13c. Experiment II Phase A2 Home Cage Activity Kruskal Wallis Test

| Kruskal-Wallis Test | Housing Condition | N | Mean Rank |
|---|---|---|---|
| **Number of Animals Moving** | Non-Enriched | 14 | 21.50 |
| | Enriched | 14 | 21.50 |
| | Super-Enriched | 14 | 21.50 |
| | Total | 42 | |
| **Amount of Activity** | Non-Enriched | 14 | 18.25 |
| | Enriched | 14 | 17.39 |
| | Super-Enriched | 14 | 28.86 |
| | Total | 42 | |
| **Level of Activity** | Non-Enriched | 14 | 13.57 |
| | Enriched | 14 | 17.07 |
| | Super-Enriched | 14 | 33.86 |
| | Total | 42 | |
| **Grouping Variable: Housing Condition** | **Number Moving** | **Activity Amount** | **Activity Level** |
| Chi-Square | .000 | 8.084 | 23.016 |
| Df | 2 | 2 | 2 |
| Asymp. Sig. | 1.000 | .018 | .000 |

## Table 14a. Experiment II Open Field MANOVA

| Dependent Variable | | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
|---|---|---|---|---|---|---|---|---|
| HACTV1 | Contrast | 312134429.056 | 2 | 156067214.528 | 21.539 | .000 | .566 | 1.000 |
| | Error | 239113003.917 | 33 | 7245848.604 | | | | |
| VACTV1 | Contrast | 1886723.389 | 2 | 943361.694 | 13.313 | .000 | .447 | .996 |
| | Error | 2338420.917 | 33 | 70861.240 | | | | |
| HACTV2 | Contrast | 981469735.509 | 2 | 490734867.754 | 47.804 | .000 | .743 | 1.000 |
| | Error | 338764221.063 | 33 | 10265582.456 | | | | |
| VACTV2 | Contrast | 4985027.722 | 2 | 2492513.861 | 28.925 | .000 | .637 | 1.000 |
| | Error | 2843622.167 | 33 | 86170.369 | | | | |
| HACTV3 | Contrast | 320948500.167 | 2 | 160474250.083 | 11.052 | .000 | .401 | .986 |
| | Error | 479153200.583 | 33 | 14519793.957 | | | | |
| VACTV3 | Contrast | 1105075.500 | 2 | 552537.750 | 3.515 | .041 | .176 | .615 |
| | Error | 5186863.250 | 33 | 157177.674 | | | | |
| HACTV4 | Contrast | 545111057.056 | 2 | 272555528.528 | 28.730 | .000 | .635 | 1.000 |
| | Error | 313063811.500 | 33 | 9486782.167 | | | | |
| VACTV4 | Contrast | 2775891.167 | 2 | 1387945.583 | 7.744 | .002 | .319 | .930 |
| | Error | 5914273.583 | 33 | 179220.412 | | | | |
| HACTV5 | Contrast | 592681121.167 | 2 | 296340560.583 | 24.658 | .000 | .599 | 1.000 |
| | Error | 396596836.833 | 33 | 12018085.965 | | | | |
| VACTV5 | Contrast | 6365604.389 | 2 | 3182802.194 | 12.984 | .000 | .440 | .995 |
| | Error | 8089353.500 | 33 | 245131.924 | | | | |
| HACTV6 | Contrast | 435084650.889 | 2 | 217542325.444 | 7.528 | .002 | .313 | .923 |
| | Error | 953575766.667 | 33 | 28896235.354 | | | | |
| VACTV6 | Contrast | 19243272.167 | 2 | 9621636.083 | 5.152 | .011 | .238 | .789 |
| | Error | 61626384.583 | 33 | 1867466.199 | | | | |

## Table 14b. Experiment II OF 1 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 114402936.239 | 11 | 10400266.931 | 63.488 | .000 | .658 | 1.000 |
| Time * Housing | 6887849.410 | 22 | 313084.064 | 1.911 | .009 | .104 | .988 |
| Error(time) | 59464666.878 | 363 | 163814.509 | | | | |
| Tests of Between -Subjects Effects | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 26011202.421 | 2 | 13005601.211 | 21.539 | .000 | .566 | 1.000 |
| Error | 19926083.660 | 33 | 603820.717 | | | | |

| Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 128.118 | 91.577 | .513 | -102.859 | 359.095 |
| | Super-Enriched | 572.625(*) | 91.577 | .000 | 341.648 | 803.602 |
| Enriched | Non-Enriched | -128.118 | 91.577 | .513 | -359.095 | 102.859 |
| | Super-Enriched | 444.507(*) | 91.577 | .000 | 213.530 | 675.484 |
| Super-Enriched | Non-Enriched | -572.625(*) | 91.577 | .000 | -803.602 | -341.648 |
| | Enriched | -444.507(*) | 91.577 | .000 | -675.484 | -213.530 |

## Table 14c. Experiment II OF 1 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 778613.189 | 11 | 70783.017 | 48.803 | .000 | .597 | 1.000 |
| Time*Housing | 64743.379 | 22 | 2942.881 | 2.029 | .004 | .110 | .992 |
| Error(time) | 526485.431 | 363 | 1450.373 | | | | |
| Tests of Between -Subjects Effects | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 157226.949 | 2 | 78613.475 | 13.313 | .000 | .447 | .996 |
| Error | 194868.410 | 33 | 5905.103 | | | | |

| Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 7.674 | 9.056 | 1.000 | -15.168 | 30.515 |
| | Super-Enriched | 43.757(*) | 9.056 | .000 | 20.915 | 66.599 |
| Enriched | Non-Enriched | -7.674 | 9.056 | 1.000 | -30.515 | 15.168 |
| | Super-Enriched | 36.083(*) | 9.056 | .001 | 13.242 | 58.925 |
| Super-Enriched | Non-Enriched | -43.757(*) | 9.056 | .000 | -66.599 | -20.915 |
| | Enriched | -36.083(*) | 9.056 | .001 | -58.925 | -13.242 |

## Table 14d. Experiment II OF 2 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 132232292.859 | 11 | 12021117.533 | 49.272 | .000 | .599 | 1.000 |
| Time*Housing | 10000051.356 | 22 | 454547.789 | 1.863 | .011 | .101 | .985 |
| Error(time) | 88562655.201 | 363 | 243974.257 | | | | |
| **Tests of Between -Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 75442886.255 | 2 | 37721443.127 | 47.870 | .000 | .744 | 1.000 |
| Error | 26004019.049 | 33 | 788000.577 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 303.757(*) | 104.616 | .020 | 39.894 | 567.620 |
| | Super-Enriched | 998.438(*) | 104.616 | .000 | 734.574 | 1262.301 |
| Enriched | Non-Enriched | -303.757(*) | 104.616 | .020 | -567.620 | -39.894 |
| | Super-Enriched | 694.681(*) | 104.616 | .000 | 430.817 | 958.544 |
| Super-Enriched | Non-Enriched | -998.438(*) | 104.616 | .000 | -1262.301 | -734.574 |
| | Enriched | -694.681(*) | 104.616 | .000 | -958.544 | -430.817 |

## Table 14e. Experiment II OF 2 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 1000437.944 | 11 | 90948.904 | 35.383 | .000 | .517 | 1.000 |
| Time*Housing | 102204.347 | 22 | 4645.652 | 1.807 | .015 | .099 | .982 |
| Error(time) | 933064.208 | 363 | 2570.425 | | | | |
| **Tests of Between -Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 413552.542 | 2 | 206776.271 | 28.744 | .000 | .635 | 1.000 |
| Error | 237392.625 | 33 | 7193.716 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 18.938 | 9.996 | .201 | -6.274 | 44.149 |
| | Super-Enriched | 73.021(*) | 9.996 | .000 | 47.810 | 98.232 |
| Enriched | Non-Enriched | -18.938 | 9.996 | .201 | -44.149 | 6.274 |
| | Super-Enriched | 54.083(*) | 9.996 | .000 | 28.872 | 79.294 |
| Super-Enriched | Non-Enriched | -73.021(*) | 9.996 | .000 | -98.232 | -47.810 |
| | Enriched | -54.083(*) | 9.996 | .000 | -79.294 | -28.872 |

## Table 14f. Experiment II OF 3 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 137746139.618 | 11 | 12522376.329 | 44.932 | .000 | .577 | 1.000 |
| Time*Housing | 7174199.264 | 22 | 326099.967 | 1.170 | .272 | .066 | .860 |
| Error(time) | 101166300.368 | 363 | 278695.042 | | | | |
| **Tests of Between -Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 26745708.347 | 2 | 13372854.174 | 11.052 | .000 | .401 | .986 |
| Error | 39929433.382 | 33 | 1209982.830 | | | | |

| Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 114.069 | 129.635 | 1.000 | -212.898 | 441.037 |
| | Super-Enriched | 575.535(*) | 129.635 | .000 | 248.567 | 902.503 |
| Enriched | Non-Enriched | -114.069 | 129.635 | 1.000 | -441.037 | 212.898 |
| | Super-Enriched | 461.465(*) | 129.635 | .003 | 134.497 | 788.433 |
| Super-Enriched | Non-Enriched | -575.535(*) | 129.635 | .000 | -902.503 | -248.567 |
| | Enriched | -461.465(*) | 129.635 | .003 | -788.433 | -134.497 |

## Table 14g. Experiment II OF 3 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 967896.019 | 11 | 87990.547 | 33.911 | .000 | .507 | 1.000 |
| Time*Housing | 83295.329 | 22 | 3786.151 | 1.459 | .085 | .081 | .941 |
| Error(time) | 941886.319 | 363 | 2594.728 | | | | |
| **Tests of Between -Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 63195.449 | 2 | 31597.725 | 2.355 | .111 | .125 | .443 |
| Error | 442751.514 | 33 | 13416.713 | | | | |

## Table 14h. Experiment II OF 4 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 162860736.546 | 11 | 14805521.504 | 60.282 | .000 | .646 | 1.000 |
| Time*Housing | 9763128.745 | 22 | 443778.579 | 1.807 | .015 | .099 | .982 |
| Error(time) | 89154933.208 | 363 | 245605.877 | | | | |

| Tests of Between -Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 45425921.421 | 2 | 22712960.711 | 28.730 | .000 | .635 | 1.000 |
| Error | 26088650.958 | 33 | 790565.181 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 136.243 | 104.786 | .608 | -128.049 | 400.535 |
| | Super-Enriched | 745.813(*) | 104.786 | .000 | 481.520 | 1010.105 |
| Enriched | Non-Enriched | -136.243 | 104.786 | .608 | -400.535 | 128.049 |
| | Super-Enriched | 609.569(*) | 104.786 | .000 | 345.277 | 873.862 |
| Super-Enriched | Non-Enriched | -745.813(*) | 104.786 | .000 | -1010.105 | -481.520 |
| | Enriched | -609.569(*) | 104.786 | .000 | -873.862 | -345.277 |

## Table 14i. Experiment II OF 4 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 1569017.951 | 11 | 142637.996 | 32.367 | .000 | .495 | 1.000 |
| Time*Housing | 175520.514 | 22 | 7978.205 | 1.810 | .015 | .099 | .982 |
| Error(time) | 1599704.451 | 363 | 4406.899 | | | | |

| Tests of Between -Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 231324.264 | 2 | 115662.132 | 7.744 | .002 | .319 | .930 |
| Error | 492856.132 | 33 | 14935.034 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 6.326 | 14.402 | 1.000 | -30.000 | 42.652 |
| | Super-Enriched | 51.944(*) | 14.402 | .003 | 15.618 | 88.271 |
| Enriched | Non-Enriched | -6.326 | 14.402 | 1.000 | -42.652 | 30.000 |
| | Super-Enriched | 45.618(*) | 14.402 | .010 | 9.292 | 81.944 |
| Super-Enriched | Non-Enriched | -51.944(*) | 14.402 | .003 | -88.271 | -15.618 |
| | Enriched | -45.618(*) | 14.402 | .010 | -81.944 | -9.292 |

## Table 14j. Experiment II OF 5 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 184771724.081 | 11 | 16797429.462 | 67.661 | .000 | .672 | 1.000 |
| Time*Housing | 8715167.370 | 22 | 396143.971 | 1.596 | .044 | .088 | .962 |
| Error(time) | 90118067.965 | 363 | 248259.140 | | | | |
| Tests of Between -Subjects Effects | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 48421653.407 | 2 | 24210826.704 | 25.648 | .000 | .609 | 1.000 |
| Error | 31150828.451 | 33 | 943964.499 | | | | |

| Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 340.667(*) | 114.502 | .016 | 51.869 | 629.464 |
| | Super-Enriched | 816.361(*) | 114.502 | .000 | 527.564 | 1105.159 |
| Enriched | Non-Enriched | -340.667(*) | 114.502 | .016 | -629.464 | -51.869 |
| | Super-Enriched | 475.694(*) | 114.502 | .001 | 186.897 | 764.492 |
| Super-Enriched | Non-Enriched | -816.361(*) | 114.502 | .000 | -1105.159 | -527.564 |
| | Enriched | -475.694(*) | 114.502 | .001 | -764.492 | -186.897 |

## Table 14k. Experiment II OF 5 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 1792026.380 | 11 | 162911.489 | 42.938 | .000 | .565 | 1.000 |
| Time*Housing | 141509.079 | 22 | 6432.231 | 1.695 | .027 | .093 | .973 |
| Error(time) | 1377246.708 | 363 | 3794.068 | | | | |
| Tests of Between -Subjects Effects | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 530467.032 | 2 | 265233.516 | 12.984 | .000 | .440 | .995 |
| Error | 674112.792 | 33 | 20427.660 | | | | |

| Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni. | | | | | | |
|---|---|---|---|---|---|---|
| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 41.215 | 16.844 | .060 | -1.269 | 83.699 |
| | Super-Enriched | 85.813(*) | 16.844 | .000 | 43.329 | 128.296 |
| Enriched | Non-Enriched | -41.215 | 16.844 | .060 | -83.699 | 1.269 |
| | Super-Enriched | 44.597(*) | 16.844 | .037 | 2.113 | 87.081 |
| Super-Enriched | Non-Enriched | -85.813(*) | 16.844 | .000 | -128.296 | -43.329 |
| | Enriched | -44.597(*) | 16.844 | .037 | -87.081 | -2.113 |

## Table 14l. Experiment II OF 6 Horizontal Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 164296458.907 | 11 | 14936041.719 | 56.915 | .000 | .633 | 1.000 |
| Time*Housing | 10045257.981 | 22 | 456602.636 | 1.740 | .021 | .095 | .977 |
| Error(time) | 95260863.611 | 363 | 262426.622 | | | | |
| **Tests of Between -Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 36257054.241 | 2 | 18128527.120 | 7.528 | .002 | .313 | .923 |
| Error | 79464647.222 | 33 | 2408019.613 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | 49.653 | 182.879 | 1.000 | -411.607 | 510.912 |
| | Super-Enriched | 637.875(*) | 182.879 | .004 | 176.615 | 1099.135 |
| Enriched | Non-Enriched | -49.653 | 182.879 | 1.000 | -510.912 | 411.607 |
| | Super-Enriched | 588.222(*) | 182.879 | .009 | 126.963 | 1049.482 |
| Super-Enriched | Non-Enriched | -637.875(*) | 182.879 | .004 | -1099.135 | -176.615 |
| | Enriched | -588.222(*) | 182.879 | .009 | -1049.482 | -126.963 |

## Table 14m. Experiment II OF 6 Vertical Activity

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 1486288.692 | 11 | 135117.154 | 37.761 | .000 | .534 | 1.000 |
| Time*Housing | 115526.134 | 22 | 5251.188 | 1.468 | .081 | .082 | .942 |
| Error(time) | 1298903.090 | 363 | 3578.245 | | | | |
| **Tests of Between -Subjects Effects** | | | | | | | |
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 439095.421 | 2 | 219547.711 | 6.497 | .004 | .283 | .879 |
| Error | 1115203.493 | 33 | 33794.045 | | | | |

**Between -Subjects Effects Post Hoc Pairwise Comparisons -** Adjustment for multiple comparisons: Bonferroni.

| (I) Housing | (J) Housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval for Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched | Enriched | -5.792 | 21.665 | 1.000 | -60.435 | 48.851 |
| | Super-Enriched | 64.549(*) | 21.665 | .016 | 9.905 | 119.192 |
| Enriched | Non-Enriched | 5.792 | 21.665 | 1.000 | -48.851 | 60.435 |
| | Super-Enriched | 70.340(*) | 21.665 | .008 | 15.697 | 124.983 |
| Super-Enriched | Non-Enriched | -64.549(*) | 21.665 | .016 | -119.192 | -9.905 |
| | Enriched | -70.340(*) | 21.665 | .008 | -124.983 | -15.697 |

**Table 15. Experiment III Timeline**

| Date | Day of Study | | Measure/Activity |
|---|---|---|---|
| 7 Apr | Day 1 - **Phase 1** | | New Housing – FC & BW |
| 8 Apr | Day 2 | | HCA |
| 9 Apr | Day 3 | | FC |
| 10 Apr | Day 4 | | |
| 11 Apr | Day 5 | | FC & BW |
| 12 Apr | Day 6 | | |
| 13 Apr | Day 7 | | HCA & FC |
| 14 Apr | Day 8 | | |
| 15 Apr | Day 9 | | FC |
| 16 Apr | Day 10 | | BW |
| 17 Apr | Day 11 | | FC |
| 18 Apr | Day 12 | | |
| 19 Apr | Day 13 | | FC & OF |
| 20 Apr | Day 14 | | HCA & BW |
| 21 Apr | Day 15 **- Phase 2** Day 1 | | FC |
| 22 Apr | Day 17 | Day 2 | HCA |
| 23 Apr | Day 18 | Day 3 | FC & OF |
| 24 Apr | Day 19 | Day 4 | |
| 25 Apr | Day 20 | Day 5 | FC & BW |
| 26 Apr | Day 21 | Day 6 | |
| 27 Apr | Day 22 | Day 7 | HCA |
| 28 Apr | Day 23 | Day 8 | |
| 29 Apr | Day 24 | Day 9 | FC |
| 30 Apr | Day 25 | Day 10 | BW |
| 1 May | Day 26 | Day 11 | FC |
| 2 May | Day 27 | Day 12 | |
| 3 May | Day 28 | Day 13 | FC & OF |
| 4 May | Day 29 | Day 14 | HCA & BW |
| 5 May | Day 30 **- Phase 3** Day 1 | | FC |
| 6 May | Day 31 | Day 2 | HCA |
| 7 May | Day 32 | Day 3 | FC & OF |
| 8 May | Day 33 | Day 4 | |
| 9 May | Day 34 | Day 5 | FC & BW |
| 10 May | Day 35 | Day 6 | |
| 11 May | Day 36 | Day 7 | HCA & FC |
| 12 May | Day 37 | Day 8 | |
| 13 May | Day 38 | Day 9 | FC |
| 14 May | Day 39 | Day 10 | BW |
| 15 May | Day 40 | Day 11 | FC |
| 16 May | Day 41 | Day 12 | |
| 17 May | Day 41 | Day 13 | FC |
| 18 May | Day 43 | Day 14 | HCA & BW |
| 19 May | Day 44 | Day 15 | FC |
| 20 May | Day 45 | Day 16 | HCA & OF |
| 21 May | Day 46 | Day 17 | FC |
| 22 May | Day 47 | Day 18 | |
| 23 May | Day 48 | Day 19 | FC & BW |
| 24 May | Day 49 | Day 20 | HCA |
| 25 May | Day 50 | Day 21 | Sacrifice |

BW = Body Weight Measurement
FC = Food Consumption
HCA = Home Cage Activity
OF = Open Field (locomotor)

## Table 16. Experiment III Body Weight ANCOVA

| Tests of Within-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Time | 7415.795 | 10 | 741.580 | 4.156 | .000 | .090 | .998 |
| Time * Housing | 21584.507 | 10 | 2158.451 | 12.096 | .000 | .224 | 1.000 |
| Time * Sex | 207205.655 | 10 | 20720.566 | 116.116 | .000 | .734 | 1.000 |
| Time*housing * Sex | 1678.393 | 10 | 167.839 | .941 | .496 | .022 | .501 |
| Error(time) | 74947.566 | 420 | 178.447 | | | | |

| Tests of Between -Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 76634.507 | 1 | 76634.507 | 15.293 | .000 | .267 | .969 |
| Sex | 401682.612 | 1 | 401682.612 | 80.161 | .000 | .656 | 1.000 |
| Housing * Sex | 1618.899 | 1 | 1618.899 | .323 | .573 | .008 | .086 |
| Error | 210461.122 | 42 | 5010.979 | | | | |

| Post-Hoc Pairwise Comparisons – No Adjustment for Multiple Comparisons | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched Males | Non-Enriched Females | 62.589(*) | 9.955 | .000 | 42.500 | 82.679 |
| | Super-Enriched Males | 21.502(*) | 9.123 | .023 | 3.092 | 39.913 |
| | Super-Enriched Females | 91.195(*) | 10.448 | .000 | 70.110 | 112.281 |
| Non-Enriched Females | Non-Enriched Males | -62.589(*) | 9.955 | .000 | -82.679 | -42.500 |
| | Super-Enriched Males | -41.087(*) | 9.059 | .000 | -59.369 | -22.805 |
| | Super-Enriched Females | 28.606(*) | 8.773 | .002 | 10.902 | 46.310 |
| Super-Enriched Males | Non-Enriched Males | -21.502(*) | 9.123 | .023 | -39.913 | -3.092 |
| | Non-Enriched Females | 41.087(*) | 9.059 | .000 | 22.805 | 59.369 |
| | Super-Enriched Females | 69.693(*) | 9.388 | .000 | 50.746 | 88.640 |
| Super-Enriched Females | Non-Enriched Males | -91.195(*) | 10.448 | .000 | -112.281 | -70.110 |
| | Non-Enriched Females | -28.606(*) | 8.773 | .002 | -46.310 | -10.902 |
| | Super-Enriched Males | -69.693(*) | 9.388 | .000 | -88.640 | -50.746 |

**Table 17. Experiment III Body Mass Index**

| Tests of Between-Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 797.011 | 1 | 797.011 | 15.458 | .000 | .264 | .970 |
| Sex | 756.578 | 1 | 756.578 | 14.674 | .000 | .254 | .963 |
| Housing * Sex | 3.204 | 1 | 3.204 | .062 | .804 | .001 | .057 |
| Error | 2217.048 | 43 | 51.559 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) ExpGrp | (J) ExpGrp | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched Males | Non-Enriched Females | 8.55259(*) | 2.997 | .032 | .54254 | 16.56264 |
| | Super-Enriched Males | 8.76437(*) | 2.997 | .027 | .75431 | 16.77442 |
| | Super-Enriched Females | 16.27180(*) | 2.997 | .000 | 8.26175 | 24.28185 |
| Non-Enriched Females | Non-Enriched Males | -8.55259(*) | 2.997 | .032 | -16.56264 | -.54254 |
| | Super-Enriched Males | .21178 | 2.931 | 1.000 | -7.62221 | 8.04576 |
| | Super-Enriched Females | 7.71921 | 2.931 | .055 | -.11478 | 15.55319 |
| Super-Enriched Males | Non-Enriched Males | -8.76437(*) | 2.997 | .027 | -16.77442 | -.75431 |
| | Non-Enriched Females | -.21178 | 2.931 | 1.000 | -8.04576 | 7.62221 |
| | Super-Enriched Females | 7.50743 | 2.931 | .065 | -.32655 | 15.34142 |
| Super-Enriched Females | Non-Enriched Males | -16.27180(*) | 2.997 | .000 | -24.28185 | -8.26175 |
| | Non-Enriched Females | -7.71921 | 2.931 | .055 | -15.55319 | .11478 |
| | Super-Enriched Males | -7.50743 | 2.931 | .065 | -15.34142 | .32655 |

## Table 18. Experiment III Lee Index Tests of Between-Subjects Effects

| Tests of Between-Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 1448.283 | 1 | 1448.283 | 8.190 | .006 | .160 | .799 |
| Sex | 474.191 | 1 | 474.191 | 2.682 | .109 | .059 | .360 |
| Housing * Sex | 76.057 | 1 | 76.057 | .430 | .515 | .010 | .098 |
| Error | 7603.976 | 43 | 176.837 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) ExpGrp | (J) ExpGrp | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Non-Enriched Males | Non-Enriched Females | -3.81120 | 5.550896 | .902 | -18.64553 | 11.02314 |
| | Super-Enriched Males | 13.65605 | 5.550896 | .081 | -1.17829 | 28.49039 |
| | Super-Enriched Females | 4.75285 | 5.550896 | .827 | -10.08149 | 19.58719 |
| Non-Enriched Females | Non-Enriched Males | 3.81120 | 5.550896 | .902 | -11.02314 | 18.64553 |
| | Super-Enriched Males | 17.46725(*) | 5.428883 | .013 | 2.95898 | 31.97552 |
| | Super-Enriched Females | 8.56405 | 5.428883 | .402 | -5.94422 | 23.07231 |
| Super-Enriched Males | Non-Enriched Males | -13.65605 | 5.550896 | .081 | -28.49039 | 1.17829 |
| | Non-Enriched Females | -17.46725(*) | 5.428883 | .013 | -31.97552 | -2.95898 |
| | Super-Enriched Females | -8.90320 | 5.428883 | .368 | -23.41147 | 5.60507 |
| Super-Enriched Females | Non-Enriched Males | -4.75285 | 5.550896 | .827 | -19.58719 | 10.08149 |
| | Non-Enriched Females | -8.56405 | 5.428883 | .402 | -23.07231 | 5.94422 |
| | Super-Enriched Males | 8.90320 | 5.428883 | .368 | -5.60507 | 23.41147 |

## Table 19a. Experiment III Phase A1 Daily Grams Consumed

| Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 89.093 | 1 | 89.093 | 69.175 | .000 | .617 | 1.000 |
| Sex | 55.985 | 1 | 55.985 | 43.469 | .000 | .503 | 1.000 |
| Housing * Sex | .142 | 1 | .142 | .110 | .742 | .003 | .062 |
| Error | 55.381 | 43 | 1.288 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | 2.0745(*) | .47372 | .000 | .8085 | 3.3404 |
| | Male Super-Enriched | 2.6456(*) | .47372 | .000 | 1.3797 | 3.9116 |
| | Female Super-Enriched | 4.9399(*) | .47372 | .000 | 3.6739 | 6.2059 |
| Female Non-Enriched | Male Non-Enriched | -2.0745(*) | .47372 | .000 | -3.3404 | -.8085 |
| | Male Super-Enriched | .5712 | .46331 | .610 | -.6670 | 1.8093 |
| | Female Super-Enriched | 2.8655(*) | .46331 | .000 | 1.6273 | 4.1036 |
| Male Super-Enriched | Male Non-Enriched | -2.6456(*) | .47372 | .000 | -3.9116 | -1.3797 |
| | Female Non-Enriched | -.5712 | .46331 | .610 | -1.8093 | .6670 |
| | Female Super-Enriched | 2.2943(*) | .46331 | .000 | 1.0561 | 3.5324 |
| Female Super-Enriched | Male Non-Enriched | -4.9399(*) | .47372 | .000 | -6.2059 | -3.6739 |
| | Female Non-Enriched | -2.8655(*) | .46331 | .000 | -4.1036 | -1.6273 |
| | Male Super-Enriched | -2.2943(*) | .46331 | .000 | -3.5324 | -1.0561 |

## Table 19b. Experiment III Phase A1 Daily Calories Consumed

| Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 38.809 | 1 | 38.809 | 69.175 | .000 | .617 | 1.000 |
| Sex | 24.387 | 1 | 24.387 | 43.469 | .000 | .503 | 1.000 |
| Housing * Sex | .062 | 1 | .062 | .110 | .742 | .003 | .062 |
| Error | 24.124 | 43 | .561 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | 1.369143(*) | .3126565 | .000 | .533593 | 2.204693 |
| | Male Super-Enriched | 1.746129(*) | .3126565 | .000 | .910578 | 2.581679 |
| | Female Super-Enriched | 3.260357(*) | .3126565 | .000 | 2.424807 | 4.095907 |
| Female Non-Enriched | Male Non-Enriched | -1.369143(*) | .3126565 | .000 | -2.204693 | -.533593 |
| | Male Super-Enriched | .376986 | .3057841 | .610 | -.440198 | 1.194170 |
| | Female Super-Enriched | 1.891214(*) | .3057841 | .000 | 1.074030 | 2.708398 |
| Male Super-Enriched | Male Non-Enriched | -1.746129(*) | .3126565 | .000 | -2.581679 | -.910578 |
| | Female Non-Enriched | -.376986 | .3057841 | .610 | -1.194170 | .440198 |
| | Female Super-Enriched | 1.514229(*) | .3057841 | .000 | .697044 | 2.331413 |
| Female Super-Enriched | Male Non-Enriched | -3.260357(*) | .3126565 | .000 | -4.095907 | -2.424807 |
| | Female Non-Enriched | -1.891214(*) | .3057841 | .000 | -2.708398 | -1.074030 |
| | Male Super-Enriched | -1.514229(*) | .3057841 | .000 | -2.331413 | -.697044 |

## Table 19c. Experiment III Phase B Daily Grams Consumed

| Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 154.180 | 1 | 154.180 | 24.173 | .000 | .360 | .998 |
| Sex | 464.934 | 1 | 464.934 | 72.894 | .000 | .629 | 1.000 |
| Housing * Sex | 13.099 | 1 | 13.099 | 2.054 | .159 | .046 | .289 |
| Error | 274.264 | 43 | 6.378 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | 5.2383(*) | 1.05421 | .000 | 2.4210 | 8.0556 |
| | Male Super-Enriched | 2.5684 | 1.05421 | .085 | -.2489 | 5.3857 |
| | Female Super-Enriched | 9.9198(*) | 1.05421 | .000 | 7.1025 | 12.7371 |
| Female Non-Enriched | Male Non-Enriched | -5.2383(*) | 1.05421 | .000 | -8.0556 | -2.4210 |
| | Male Super-Enriched | -2.6699 | 1.03104 | .061 | -5.4252 | .0855 |
| | Female Super-Enriched | 4.6815(*) | 1.03104 | .000 | 1.9262 | 7.4369 |
| Male Super-Enriched | Male Non-Enriched | -2.5684 | 1.05421 | .085 | -5.3857 | .2489 |
| | Female Non-Enriched | 2.6699 | 1.03104 | .061 | -.0855 | 5.4252 |
| | Female Super-Enriched | 7.3514(*) | 1.03104 | .000 | 4.5961 | 10.1068 |
| Female Super-Enriched | Male Non-Enriched | -9.9198(*) | 1.05421 | .000 | -12.7371 | -7.1025 |
| | Female Non-Enriched | -4.6815(*) | 1.03104 | .000 | -7.4369 | -1.9262 |
| | Male Super-Enriched | -7.3514(*) | 1.03104 | .000 | -10.1068 | -4.5961 |

## Table 19d. Experiment III Phase B Daily Calories Consumed

| | Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 3746.951 | 1 | 3746.951 | 40.851 | .000 | .487 | 1.000 |
| Sex | 398.260 | 1 | 398.260 | 4.342 | .043 | .092 | .531 |
| Housing * Sex | .647 | 1 | .647 | .007 | .933 | .000 | .051 |
| Error | 3944.101 | 43 | 91.723 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | 5.591212 | 3.9977603 | .507 | -5.092492 | 16.274915 |
| | Male Super-Enriched | 17.635337(*) | 3.9977603 | .000 | 6.951634 | 28.319041 |
| | Female Super-Enriched | 23.696181(*) | 3.9977603 | .000 | 13.012477 | 34.379884 |
| Female Non-Enriched | Male Non-Enriched | -5.591212 | 3.9977603 | .507 | -16.274915 | 5.092492 |
| | Male Super-Enriched | 12.044126(*) | 3.9098867 | .018 | 1.595258 | 22.492994 |
| | Female Super-Enriched | 18.104969(*) | 3.9098867 | .000 | 7.656101 | 28.553837 |
| Male Super-Enriched | Male Non-Enriched | -17.635337(*) | 3.9977603 | .000 | -28.319041 | -6.951634 |
| | Female Non-Enriched | -12.044126(*) | 3.9098867 | .018 | -22.492994 | -1.595258 |
| | Female Super-Enriched | 6.060843 | 3.9098867 | .417 | -4.388025 | 16.509711 |
| Female Super-Enriched | Male Non-Enriched | -23.696181(*) | 3.9977603 | .000 | -34.379884 | -13.012477 |
| | Female Non-Enriched | -18.104969(*) | 3.9098867 | .000 | -28.553837 | -7.656101 |
| | Male Super-Enriched | -6.060843 | 3.9098867 | .417 | -16.509711 | 4.388025 |

## Table 19e. Experiment III Phase B Daily Standard Chow Grams Consumed

| | Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | .001 | 1 | .001 | .000 | .990 | .000 | .050 |
| Sex | 439.339 | 1 | 439.339 | 150.316 | .000 | .778 | 1.000 |
| Housing * Sex | 14.610 | 1 | 14.610 | 4.999 | .031 | .104 | .589 |
| Error | 125.679 | 43 | 2.923 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | 5.0032(*) | .71363 | .000 | 3.0961 | 6.9104 |
| | Male Super-Enriched | -1.1225 | .71363 | .404 | -3.0296 | .7847 |
| | Female Super-Enriched | 6.1125(*) | .71363 | .000 | 4.2054 | 8.0197 |
| Female Non-Enriched | Male Non-Enriched | -5.0032(*) | .71363 | .000 | -6.9104 | -3.0961 |
| | Male Super-Enriched | -6.1257(*) | .69795 | .000 | -7.9909 | -4.2605 |
| | Female Super-Enriched | 1.1093 | .69795 | .395 | -.7559 | 2.9745 |
| Male Super-Enriched | Male Non-Enriched | 1.1225 | .71363 | .404 | -.7847 | 3.0296 |
| | Female Non-Enriched | 6.1257(*) | .69795 | .000 | 4.2605 | 7.9909 |
| | Female Super-Enriched | 7.2350(*) | .69795 | .000 | 5.3698 | 9.1002 |
| Female Super-Enriched | Male Non-Enriched | -6.1125(*) | .71363 | .000 | -8.0197 | -4.2054 |
| | Female Non-Enriched | -1.1093 | .69795 | .395 | -2.9745 | .7559 |
| | Male Super-Enriched | -7.2350(*) | .69795 | .000 | -9.1002 | -5.3698 |

## Table 19f. Experiment III Phase B Daily Standard Chow Calories Consumed

| Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | .000 | 1 | .000 | .000 | .990 | .000 | .050 |
| Sex | 191.376 | 1 | 191.376 | 150.316 | .000 | .778 | 1.000 |
| Housing * Sex | 6.364 | 1 | 6.364 | 4.999 | .031 | .104 | .589 |
| Error | 54.746 | 43 | 1.273 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | 3.30214(*) | .470998 | .000 | 2.04344 | 4.56085 |
| | Male Super-Enriched | -.74083 | .470998 | .404 | -1.99953 | .51788 |
| | Female Super-Enriched | 4.03427(*) | .470998 | .000 | 2.77557 | 5.29298 |
| Female Non-Enriched | Male Non-Enriched | -3.30214(*) | .470998 | .000 | -4.56085 | -2.04344 |
| | Male Super-Enriched | -4.04297(*) | .460645 | .000 | -5.27401 | -2.81193 |
| | Female Super-Enriched | .73213 | .460645 | .395 | -.49891 | 1.96317 |
| Male Super-Enriched | Male Non-Enriched | .74083 | .470998 | .404 | -.51788 | 1.99953 |
| | Female Non-Enriched | 4.04297(*) | .460645 | .000 | 2.81193 | 5.27401 |
| | Female Super-Enriched | 4.77510(*) | .460645 | .000 | 3.54406 | 6.00614 |
| Female Super-Enriched | Male Non-Enriched | -4.03427(*) | .470998 | .000 | -5.29298 | -2.77557 |
| | Female Non-Enriched | -.73213 | .460645 | .395 | -1.96317 | .49891 |
| | Male Super-Enriched | -4.77510(*) | .460645 | .000 | -6.00614 | -3.54406 |

## Table 19g. Experiment III Phase B Daily Cookie Grams Consumed

| Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 26.180 | 1 | 26.180 | 17.454 | .000 | .289 | .983 |
| Sex | 3.478 | 1 | 3.478 | 2.319 | .135 | .051 | .319 |
| Housing * Sex | 2.935 | 1 | 2.935 | 1.957 | .169 | .044 | .277 |
| Error | 64.499 | 43 | 1.500 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | -1.0446 | .51123 | .188 | -2.4109 | .3216 |
| | Male Super-Enriched | .9936 | .51123 | .226 | -.3727 | 2.3598 |
| | Female Super-Enriched | .9493 | .51123 | .262 | -.4169 | 2.3155 |
| Female Non-Enriched | Male Non-Enriched | 1.0446 | .51123 | .188 | -.3216 | 2.4109 |
| | Male Super-Enriched | 2.0382(*) | .50000 | .001 | .7020 | 3.3744 |
| | Female Super-Enriched | 1.9939(*) | .50000 | .001 | .6577 | 3.3301 |
| Male Super-Enriched | Male Non-Enriched | -.9936 | .51123 | .226 | -2.3598 | .3727 |
| | Female Non-Enriched | -2.0382(*) | .50000 | .001 | -3.3744 | -.7020 |
| | Female Super-Enriched | -.0443 | .50000 | 1.000 | -1.3805 | 1.2919 |
| Female Super-Enriched | Male Non-Enriched | -.9493 | .51123 | .262 | -2.3155 | .4169 |
| | Female Non-Enriched | -1.9939(*) | .50000 | .001 | -3.3301 | -.6577 |
| | Male Super-Enriched | .0443 | .50000 | 1.000 | -1.2919 | 1.3805 |

## Table 19h. Experiment III Phase B Daily Cookie Calories Consumed

| Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 816.083 | 1 | 816.083 | 25.938 | .000 | .376 | .999 |
| Sex | 113.326 | 1 | 113.326 | 3.602 | .064 | .077 | .458 |
| Housing * Sex | 54.247 | 1 | 54.247 | 1.724 | .196 | .039 | .250 |
| Error | 1352.915 | 43 | 31.463 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | -5.258010 | 2.3414124 | .127 | -11.5152 | .999232 |
| | Male Super-Enriched | 6.189620 | 2.3414124 | .053 | -.067622 | 12.446863 |
| | Female Super-Enriched | 5.232004 | 2.3414124 | .130 | -1.02523 | 11.489246 |
| Female Non-Enriched | Male Non-Enriched | 5.258010 | 2.3414124 | .127 | -.999232 | 11.515253 |
| | Male Super-Enriched | 11.447631(*) | 2.2899465 | .000 | 5.327927 | 17.567334 |
| | Female Super-Enriched | 10.490014(*) | 2.2899465 | .000 | 4.370310 | 16.609718 |
| Male Super-Enriched | Male Non-Enriched | -6.189620 | 2.3414124 | .053 | -12.4468 | .067622 |
| | Female Non-Enriched | -11.447631(*) | 2.2899465 | .000 | -17.5673 | -5.327927 |
| | Female Super-Enriched | -.957617 | 2.2899465 | .975 | -7.07732 | 5.162087 |
| Female Super-Enriched | Male Non-Enriched | -5.232004 | 2.3414124 | .130 | -11.4892 | 1.025239 |
| | Female Non-Enriched | -10.490014(*) | 2.2899465 | .000 | -16.6097 | -4.370310 |
| | Male Super-Enriched | .957617 | 2.2899465 | .975 | -5.1620 | 7.077321 |

## Table 19i. Experiment III Phase B Daily Chip Grams Consumed

| Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 61.317 | 1 | 61.317 | 21.237 | .000 | .326 | .995 |
| Sex | 8.525 | 1 | 8.525 | 2.953 | .093 | .063 | .390 |
| Housing * Sex | 5.584 | 1 | 5.584 | 1.934 | .171 | .042 | .275 |
| Error | 127.040 | 44 | 2.887 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | 1.5250 | .69370 | .140 | -.3272 | 3.3772 |
| | Male Super-Enriched | 2.9426(*) | .69370 | .001 | 1.0904 | 4.7948 |
| | Female Super-Enriched | 3.1033(*) | .69370 | .000 | 1.2512 | 4.9555 |
| Female Non-Enriched | Male Non-Enriched | -1.5250 | .69370 | .140 | -3.3772 | .3272 |
| | Male Super-Enriched | 1.4176 | .69370 | .188 | -.4346 | 3.2698 |
| | Female Super-Enriched | 1.5783 | .69370 | .120 | -.2738 | 3.4305 |
| Male Super-Enriched | Male Non-Enriched | -2.9426(*) | .69370 | .001 | -4.7948 | -1.0904 |
| | Female Non-Enriched | -1.4176 | .69370 | .188 | -3.2698 | .4346 |
| | Female Super-Enriched | .1607 | .69370 | .996 | -1.6915 | 2.0129 |
| Female Super-Enriched | Male Non-Enriched | -3.1033(*) | .69370 | .000 | -4.9555 | -1.2512 |
| | Female Non-Enriched | -1.5783 | .69370 | .120 | -3.4305 | .2738 |
| | Male Super-Enriched | -.1607 | .69370 | .996 | -2.0129 | 1.6915 |

## Table 19j. Experiment III Phase B Daily Chip Calories Consumed

| Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 1066.680 | 1 | 1066.680 | 16.437 | .000 | .277 | .977 |
| Sex | 281.168 | 1 | 281.168 | 4.333 | .043 | .092 | .530 |
| Housing * Sex | 82.513 | 1 | 82.513 | 1.271 | .266 | .029 | .197 |
| Error | 2790.474 | 43 | 64.895 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | 7.54708 | 3.362650 | .128 | -1.43934 | 16.53350 |
| | Male Super-Enriched | 12.18655(*) | 3.362650 | .004 | 3.20012 | 21.17297 |
| | Female Super-Enriched | 14.42991(*) | 3.362650 | .001 | 5.44348 | 23.41633 |
| Female Non-Enriched | Male Non-Enriched | -7.54708 | 3.362650 | .128 | -16.53350 | 1.43934 |
| | Male Super-Enriched | 4.63947 | 3.288737 | .500 | -4.14943 | 13.42836 |
| | Female Super-Enriched | 6.88283 | 3.288737 | .172 | -1.90607 | 15.67172 |
| Male Super-Enriched | Male Non-Enriched | -12.18655(*) | 3.362650 | .004 | -21.17297 | -3.20012 |
| | Female Non-Enriched | -4.63947 | 3.288737 | .500 | -13.42836 | 4.14943 |
| | Female Super-Enriched | 2.24336 | 3.288737 | .903 | -6.54553 | 11.03225 |
| Female Super-Enriched | Male Non-Enriched | -14.42991(*) | 3.362650 | .001 | -23.41633 | -5.44348 |
| | Female Non-Enriched | -6.88283 | 3.288737 | .172 | -15.67172 | 1.90607 |
| | Male Super-Enriched | -2.24336 | 3.288737 | .903 | -11.03225 | 6.54553 |

## Table 19k. Experiment III Phase A2 Daily Grams Consumed

| Tests of Between -Subjects Effects | | | | | | |
|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 156.022 | 1 | 156.022 | 59.788 | .000 | .582 | 1.000 |
| Sex | 768.156 | 1 | 768.156 | 294.360 | .000 | .873 | 1.000 |
| Housing * Sex | 6.164 | 1 | 6.164 | 2.362 | .132 | .052 | .324 |
| Error | 112.212 | 43 | 2.610 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | 7.3664(*) | .67431 | .000 | 5.5644 | 9.1685 |
| | Male Super-Enriched | 2.9218(*) | .67431 | .000 | 1.1197 | 4.7238 |
| | Female Super-Enriched | 11.7378(*) | .67431 | .000 | 9.9357 | 13.5398 |
| Female Non-Enriched | Male Non-Enriched | -7.3664(*) | .67431 | .000 | -9.1685 | -5.5644 |
| | Male Super-Enriched | -4.4447(*) | .65949 | .000 | -6.2071 | -2.6822 |
| | Female Super-Enriched | 4.3713(*) | .65949 | .000 | 2.6089 | 6.1338 |
| Male Super-Enriched | Male Non-Enriched | -2.9218(*) | .67431 | .000 | -4.7238 | -1.1197 |
| | Female Non-Enriched | 4.4447(*) | .65949 | .000 | 2.6822 | 6.2071 |
| | Female Super-Enriched | 8.8160(*) | .65949 | .000 | 7.0536 | 10.5784 |
| Female Super-Enriched | Male Non-Enriched | -11.7378(*) | .67431 | .000 | -13.5398 | -9.9357 |
| | Female Non-Enriched | -4.3713(*) | .65949 | .000 | -6.1338 | -2.6089 |
| | Male Super-Enriched | -8.8160(*) | .65949 | .000 | -10.5784 | -7.0536 |

## Table 19l. Experiment III Phase A2 Daily Calories Consumed

| Tests of Between -Subjects Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
| Housing | 67.963 | 1 | 67.963 | 59.788 | .000 | .582 | 1.000 |
| Sex | 334.609 | 1 | 334.609 | 294.360 | .000 | .873 | 1.000 |
| Housing * Sex | 2.685 | 1 | 2.685 | 2.362 | .132 | .052 | .324 |
| Error | 48.880 | 43 | 1.137 | | | | |

| Post-Hoc Analyses - Tukey HSD | | | | | | |
|---|---|---|---|---|---|---|
| (I) Expgrp | (J) Expgrp | Mean Difference (I-J) | Std. Error | Sig | 95% Confidence Interval for Difference | |
| | | | | | Lower Bound | Upper Bound |
| Male Non-Enriched | Female Non-Enriched | 4.86185(*) | .445047 | .000 | 3.67250 | 6.05120 |
| | Male Super-Enriched | 1.92837(*) | .445047 | .000 | .73902 | 3.11772 |
| | Female Super-Enriched | 7.74693(*) | .445047 | .000 | 6.55758 | 8.93628 |
| Female Non-Enriched | Male Non-Enriched | -4.86185(*) | .445047 | .000 | -6.05120 | -3.67250 |
| | Male Super-Enriched | -2.93348(*) | .435265 | .000 | -4.09669 | -1.77027 |
| | Female Super-Enriched | 2.88508(*) | .435265 | .000 | 1.72187 | 4.04829 |
| Male Super-Enriched | Male Non-Enriched | -1.92837(*) | .445047 | .000 | -3.11772 | -.73902 |
| | Female Non-Enriched | 2.93348(*) | .435265 | .000 | 1.77027 | 4.09669 |
| | Female Super-Enriched | 5.81856(*) | .435265 | .000 | 4.65535 | 6.98177 |
| Female Super-Enriched | Male Non-Enriched | -7.74693(*) | .445047 | .000 | -8.93628 | -6.55758 |
| | Female Non-Enriched | -2.88508(*) | .435265 | .000 | -4.04829 | -1.72187 |
| | Male Super-Enriched | -5.81856(*) | .435265 | .000 | -6.98177 | -4.65535 |

## Table 20a. Experiment III Phase A1 Home Cage Activity Kruskal-Wallis

| Kruskal-Wallis Test | Housing Condition | N | Mean Rank |
|---|---|---|---|
| **Number Moving** | Non-Enriched Male | 9 | 17.17 |
| | Non-Enriched Female | 9 | 14.61 |
| | Super-Enriched Male | 9 | 18.94 |
| | Super-Enriched Female | 9 | 23.28 |
| | Total | 36 | |
| **Amount Activity** | Non-Enriched Male | 9 | 12.83 |
| | Non-Enriched Female | 9 | 12.67 |
| | Super-Enriched Male | 9 | 26.67 |
| | Super-Enriched Female | 9 | 21.83 |
| | Total | 36 | |
| **Level Activity** | Non-Enriched Male | 9 | 13.67 |
| | Non-Enriched Female | 9 | 13.50 |
| | Super-Enriched Male | 9 | 27.22 |
| | Super-Enriched Female | 9 | 19.61 |
| | Total | 36 | |

| Grouping Variable: Housing Condition | Number Moving | Activity Amount | Activity Level |
|---|---|---|---|
| Chi-Square | 3.589 | 12.470 | 11.070 |
| df | 3 | 3 | 3 |
| Asymp. Sig. | .309 | .006 | .011 |

## Table 20b. Experiment III Phase B Home Cage Activity Kruskal-Wallis

| Kruskal-Wallis Test | Housing Condition | N | Mean Rank |
|---|---|---|---|
| Number Moving | Non-Enriched Male | 12 | 15.50 |
| | Non-Enriched Female | 14 | 27.50 |
| | Super-Enriched Male | 10 | 27.50 |
| | Super-Enriched Female | 12 | 27.50 |
| | Total | 48 | |
| Amount Activity | Non-Enriched Male | 12 | 17.63 |
| | Non-Enriched Female | 14 | 15.54 |
| | Super-Enriched Male | 10 | 30.00 |
| | Super-Enriched Female | 12 | 37.25 |
| | Total | 48 | |
| Level Activity | Non-Enriched Male | 12 | 16.25 |
| | Non-Enriched Female | 14 | 18.11 |
| | Super-Enriched Male | 10 | 30.25 |
| | Super-Enriched Female | 12 | 35.42 |
| | Total | 48 | |

| Grouping Variable: Housing Condition | Number Moving | Activity Amount | Activity Level |
|---|---|---|---|
| Chi-Square | 20.143 | 22.786 | 17.464 |
| df | 3 | 3 | 3 |
| Asymp. Sig. | .000 | .000 | .001 |

## Table 20c. Experiment III Phase A2 Home Cage Activity Kruskal-Wallis

| Kruskal-Wallis Test | Housing Condition | N | Mean Rank |
|---|---|---|---|
| Number Moving | Non-Enriched Male | 16 | 31.19 |
| | Non-Enriched Female | 16 | 35.00 |
| | Super-Enriched Male | 16 | 31.19 |
| | Super-Enriched Female | 15 | 30.53 |
| | Total | 63 | |
| Amount Activity | Non-Enriched Male | 16 | 20.38 |
| | Non-Enriched Female | 16 | 19.38 |
| | Super-Enriched Male | 16 | 39.56 |
| | Super-Enriched Female | 15 | 49.80 |
| | Total | 63 | |
| Level Activity | Non-Enriched Male | 16 | 22.63 |
| | Non-Enriched Female | 16 | 16.75 |
| | Super-Enriched Male | 16 | 39.97 |
| | Super-Enriched Female | 15 | 49.77 |
| | Total | 63 | |

| Grouping Variable: Housing Condition | Number Moving | Activity Amount | Activity Level |
|---|---|---|---|
| Chi-Square | 2.267 | 33.625 | 34.558 |
| df | 3 | 3 | 3 |
| Asymp. Sig. | .519 | .000 | .000 |

**Table 21a. Experiment III Open Field Activity Between Subjects MANOVA**

| Source | Dependent Variable | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Power |
|---|---|---|---|---|---|---|---|---|
| Sex | HACTV1 | 87300.334 | 1 | 87300.334 | .013 | .911 | .000 | .051 |
| | VACTV1 | 5114.839 | 1 | 5114.839 | .132 | .718 | .003 | .065 |
| | HACTV2 | 40154044.512 | 1 | 40154044.512 | 2.704 | .107 | .059 | .362 |
| | VACTV2 | 69409.600 | 1 | 69409.600 | .511 | .479 | .012 | .108 |
| | HACTV3 | 27727344.089 | 1 | 27727344.089 | 1.745 | .194 | .039 | .252 |
| | VACTV3 | 960070.251 | 1 | 960070.251 | 4.083 | .050 | .087 | .506 |
| Housing | HACTV1 | 1675547208.890 | 1 | 1675547208.890 | 242.947 | .000 | .850 | 1.000 |
| | VACTV1 | 4397587.691 | 1 | 4397587.691 | 113.804 | .000 | .726 | 1.000 |
| | HACTV2 | 1443860935.890 | 1 | 1443860935.890 | 97.225 | .000 | .693 | 1.000 |
| | VACTV2 | 6167397.778 | 1 | 6167397.778 | 45.398 | .000 | .514 | 1.000 |
| | HACTV3 | 1397771021.111 | 1 | 1397771021.111 | 87.960 | .000 | .672 | 1.000 |
| | VACTV3 | 16883309.051 | 1 | 16883309.051 | 71.806 | .000 | .625 | 1.000 |
| Sex * Housing | HACTV1 | 338066.556 | 1 | 338066.556 | .049 | .826 | .001 | .055 |
| | VACTV1 | 53004.972 | 1 | 53004.972 | 1.372 | .248 | .031 | .209 |
| | HACTV2 | 4248568.956 | 1 | 4248568.956 | .286 | .595 | .007 | .082 |
| | VACTV2 | 219699.600 | 1 | 219699.600 | 1.617 | .210 | .036 | .237 |
| | HACTV3 | 5833201.422 | 1 | 5833201.422 | .367 | .548 | .008 | .091 |
| | VACTV3 | 1208757.614 | 1 | 1208757.614 | 5.141 | .028 | .107 | .601 |
| Error | HACTV1 | 296561000.136 | 43 | 6896767.445 | | | | |
| | VACTV1 | 1661598.742 | 43 | 38641.831 | | | | |
| | HACTV2 | 638578040.303 | 43 | 14850652.100 | | | | |
| | VACTV2 | 5841606.826 | 43 | 135851.322 | | | | |
| | HACTV3 | 683309874.326 | 43 | 15890927.310 | | | | |
| | VACTV3 | 10110342.886 | 43 | 235124.253 | | | | |
| | HACTV3 | 2121217145.106 | 46 | | | | | |
| | VACTV3 | 28762204.213 | 46 | | | | | |

**Table 21b. Experiment III Open Field Activity Between Subjects Tukey HSD Post-Hoc Comparisons Continued**

| Variable | (I) housing | (J) housing | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| VACTV2 | Non-Enriched Males | Non-Enriched Females | 59.924 | 153.8540 | .980 | -351.239 | 471.087 |
| | | Super-Enriched Males | 861.841(*) | 153.8540 | .000 | 450.678 | 1273.004 |
| | | Super-Enriched Females | 648.091(*) | 153.8540 | .001 | 236.928 | 1059.254 |
| | Non-Enriched Females | Non-Enriched Males | -59.924 | 153.8540 | .980 | -471.087 | 351.239 |
| | | Super-Enriched Males | 801.917(*) | 150.4722 | .000 | 399.791 | 1204.042 |
| | | Super-Enriched Females | 588.167(*) | 150.4722 | .002 | 186.041 | 990.292 |
| | Super-Enriched Males | Non-Enriched Males | -861.841(*) | 153.8540 | .000 | -1273.004 | -450.678 |
| | | Non-Enriched Females | -801.917(*) | 150.4722 | .000 | -1204.042 | -399.791 |
| | | Super-Enriched Females | -213.750 | 150.4722 | .494 | -615.875 | 188.375 |
| | Super-Enriched Females | Non-Enriched Males | -648.091(*) | 153.8540 | .001 | -1059.254 | -236.928 |
| | | Non-Enriched Females | -588.167(*) | 150.4722 | .002 | -990.292 | -186.041 |
| | | Super-Enriched Males | 213.750 | 150.4722 | .494 | -188.375 | 615.875 |
| HACTV3 | Non-Enriched Males | Non-Enriched Females | -832.159 | 1663.9933 | .959 | -5279.052 | 3614.734 |
| | | Super-Enriched Males | 11619.674(*) | 1663.9933 | .000 | 7172.782 | 16066.567 |
| | | Super-Enriched Females | 9377.341(*) | 1663.9933 | .000 | 4930.448 | 13824.234 |
| | Non-Enriched Females | Non-Enriched Males | 832.159 | 1663.9933 | .959 | -3614.734 | 5279.052 |
| | | Super-Enriched Males | 12451.833(*) | 1627.4176 | .000 | 8102.687 | 16800.980 |
| | | Super-Enriched Females | 10209.500(*) | 1627.4176 | .000 | 5860.353 | 14558.647 |
| | Super-Enriched Males | Non-Enriched Males | -11619.674(*) | 1663.9933 | .000 | -16066.567 | -7172.782 |
| | | Non-Enriched Females | -12451.833(*) | 1627.4176 | .000 | -16800.980 | -8102.687 |
| | | Super-Enriched Females | -2242.333 | 1627.4176 | .520 | -6591.480 | 2106.813 |
| | Super-Enriched Females | Non-Enriched Males | -9377.341(*) | 1663.9933 | .000 | -13824.234 | -4930.448 |
| | | Non-Enriched Females | -10209.500(*) | 1627.4176 | .000 | -14558.647 | -5860.353 |
| | | Super-Enriched Males | 2242.333 | 1627.4176 | .520 | -2106.813 | 6591.480 |
| VACTV3 | Non-Enriched Males | Non-Enriched Females | 607.015(*) | 202.4071 | .022 | 66.098 | 1147.932 |
| | | Super-Enriched Males | 1520.515(*) | 202.4071 | .000 | 979.598 | 2061.432 |
| | | Super-Enriched Females | 1485.598(*) | 202.4071 | .000 | 944.681 | 2026.516 |
| | Non-Enriched Females | Non-Enriched Males | -607.015(*) | 202.4071 | .022 | -1147.932 | -66.098 |
| | | Super-Enriched Males | 913.500(*) | 197.9580 | .000 | 384.473 | 1442.527 |
| | | Super-Enriched Females | 878.583(*) | 197.9580 | .000 | 349.556 | 1407.611 |
| | Super-Enriched Males | Non-Enriched Males | -1520.515(*) | 202.4071 | .000 | -2061.432 | -979.598 |
| | | Non-Enriched Females | -913.500(*) | 197.9580 | .000 | -1442.527 | -384.473 |
| | | Super-Enriched Females | -34.917 | 197.9580 | .998 | -563.944 | 494.111 |
| | Super-Enriched Females | Non-Enriched Males | -1485.598(*) | 202.4071 | .000 | -2026.516 | -944.681 |
| | | Non-Enriched Females | -878.583(*) | 197.9580 | .000 | -1407.611 | -349.556 |
| | | Super-Enriched Males | 34.917 | 197.9580 | .998 | -494.111 | 563.944 |

**Table 21c. Experiment III Open Field Activity Tests of Between-Subjects Effects**

| Variables | Dependent Variable | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
|---|---|---|---|---|---|---|---|---|
| Sex | HACTV1 | 87300.334 | 1 | 87300.334 | .013 | .911 | .000 | .051 |
| | VACTV1 | 5114.839 | 1 | 5114.839 | .132 | .718 | .003 | .065 |
| | HACTV2 | 40154044.512 | 1 | 40154044.512 | 2.704 | .107 | .059 | .362 |
| | VACTV2 | 69409.600 | 1 | 69409.600 | .511 | .479 | .012 | .108 |
| | HACTV3 | 27727344.089 | 1 | 27727344.089 | 1.745 | .194 | .039 | .252 |
| | VACTV3 | 960070.251 | 1 | 960070.251 | 4.083 | .050 | .087 | .506 |
| Housing | HACTV1 | 1675547208.890 | 1 | 1675547208.890 | 242.947 | .000 | .850 | 1.000 |
| | VACTV1 | 4397587.691 | 1 | 4397587.691 | 113.804 | .000 | .726 | 1.000 |
| | HACTV2 | 1443860935.890 | 1 | 1443860935.890 | 97.225 | .000 | .693 | 1.000 |
| | VACTV2 | 6167397.778 | 1 | 6167397.778 | 45.398 | .000 | .514 | 1.000 |
| | HACTV3 | 1397771021.111 | 1 | 1397771021.111 | 87.960 | .000 | .672 | 1.000 |
| | VACTV3 | 16883309.051 | 1 | 16883309.051 | 71.806 | .000 | .625 | 1.000 |
| Sex * Housing | HACTV1 | 338066.556 | 1 | 338066.556 | .049 | .826 | .001 | .055 |
| | VACTV1 | 53004.972 | 1 | 53004.972 | 1.372 | .248 | .031 | .209 |
| | HACTV2 | 4248568.956 | 1 | 4248568.956 | .286 | .595 | .007 | .082 |
| | VACTV2 | 219699.600 | 1 | 219699.600 | 1.617 | .210 | .036 | .237 |
| | HACTV3 | 5833201.422 | 1 | 5833201.422 | .367 | .548 | .008 | .091 |
| | VACTV3 | 1208757.614 | 1 | 1208757.614 | 5.141 | .028 | .107 | .601 |
| Error | HACTV1 | 296561000.136 | 43 | 6896767.445 | | | | |
| | VACTV1 | 1661598.742 | 43 | 38641.831 | | | | |
| | HACTV2 | 638578040.303 | 43 | 14850652.100 | | | | |
| | VACTV2 | 5841606.826 | 43 | 135851.322 | | | | |
| | HACTV3 | 683309874.326 | 43 | 15890927.310 | | | | |
| | VACTV3 | 10110342.886 | 43 | 235124.253 | | | | |

## Table 21d. Experiment III OF Activity Within-Subjects Tests of Effects

| Variable | Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Observed Power |
|---|---|---|---|---|---|---|---|
| **OF 1 HACT** Time | 179062982.355 | 11 | 16278452.941 | 78.060 | .000 | .645 | 1.000 |
| **OF 1 VACT** Time | 835388.752 | 11 | 75944.432 | 58.025 | .000 | .574 | 1.000 |
| **OF 2 HACT** Time | 198650293.636 | 11 | 18059117.603 | 65.474 | .000 | .604 | 1.000 |
| **OF 2 VACT** Time | 1645486.979 | 11 | 149589.725 | 53.879 | .000 | .556 | 1.000 |
| **OF 3 HACT** Time | 148086744.719 | 11 | 13462431.338 | 63.529 | .000 | .596 | 1.000 |
| **OF 3 VACT** Time | 1624852.810 | 11 | 147713.892 | 37.862 | .000 | .468 | 1.000 |
| **OF 1 HACT** Time * Sex | 1869298.898 | 11 | 169936.263 | .815 | .625 | .019 | .459 |
| **OF 1 VACT** Time * Sex | 23444.134 | 11 | 2131.285 | 1.628 | .088 | .036 | .821 |
| **OF 2 HACT** Time * Sex | 2526452.874 | 11 | 229677.534 | .833 | .607 | .019 | .469 |
| **OF 2 VACT** Time * Sex | 39339.538 | 11 | 3576.322 | 1.288 | .228 | .029 | .700 |
| **OF 3 HACT** Time * Sex | 3497889.717 | 11 | 317989.974 | 1.501 | .128 | .034 | .781 |
| **OF 3 VACT** Time * Sex | 54684.457 | 11 | 4971.314 | 1.274 | .236 | .029 | .694 |
| **OF 1 HACT** Time * Housing | 49429383.276 | 11 | 4493580.298 | 21.548 | .000 | .334 | 1.000 |
| **OF 1 VACT** Time * Housing | 150164.234 | 11 | 13651.294 | 10.430 | .000 | .195 | 1.000 |
| **OF 2 HACT** Time * Housing | 25236562.674 | 11 | 2294232.970 | 8.318 | .000 | .162 | 1.000 |
| **OF 2 VACT** Time * Housing | 157702.146 | 11 | 14336.559 | 5.164 | .000 | .107 | 1.000 |
| **OF 3 HACT** Time * Housing | 14348871.532 | 11 | 1304442.867 | 6.156 | .000 | .125 | 1.000 |
| **OF 3 VACT** Time * Housing | 102818.176 | 11 | 9347.107 | 2.396 | .007 | .053 | .956 |
| **OF 1 HACT** Time * Sex * Housing | 3597243.231 | 11 | 327022.112 | 1.568 | .105 | .035 | .803 |
| **OF 1 VACT** Time * Sex * Housing | 22306.341 | 11 | 2027.849 | 1.549 | .111 | .035 | .797 |
| **OF 2 HACT** Time * Sex * Housing | 3077974.740 | 11 | 279815.885 | 1.014 | .432 | .023 | .569 |
| **OF 2 VACT** Time * Sex * Housing | 68168.816 | 11 | 6197.165 | 2.232 | .012 | .049 | .939 |
| **OF 3 HACT** Time * Sex * Housing | 3830385.289 | 11 | 348216.844 | 1.643 | .084 | .037 | .825 |
| **OF 3 VACT** Time * Sex * Housing | 74702.659 | 11 | 6791.151 | 1.741 | .062 | .039 | .851 |
| **OF 1 HACT** Error(Time) | 98638596.502 | 473 | 208538.259 | | | | |
| **OF 1 VACT** Error(Time) | 619075.908 | 473 | 1308.829 | | | | |
| **OF 2 HACT** Error(Time) | 130464289.581 | 473 | 275823.022 | | | | |
| **OF 2 VACT** Error(Time) | 1313229.878 | 473 | 2776.385 | | | | |
| **OF 3 HACT** Error(Time) | 100232720.176 | 473 | 211908.499 | | | | |
| **OF 3 VACT** Error(Time) | 1845333.552 | 473 | 3901.339 | | | | |

**Table 22. Mean Body Lengths For All Three Experiments**

| Experiment I (Males) | Mean Body Length | Standard Deviation |
|---|---|---|
| Non-Enriched | 23.13 | 1.07 |
| Enriched | 23.73 | 0.66 |
| Super-Enriched | 23.14 | 1.02 |
| Lengths were not significantly different | | |
| Experiment II (Females) | | |
| Non-Enriched | 19.51 | 1.01 |
| Enriched | 19.09 | 0.57 |
| Super-Enriched | 19.30 | 0.60 |
| Lengths were not significantly different | | |
| Experiment III (Males & Females) | | |
| Males Non-Enriched | 23.77 | 0.81 |
| Males Super-Enriched | 23.78 | 1.09 |
| Females Non-Enriched | 20.19 | 1.17 |
| Females Super-Enriched | 19.4 | 0.71 |
| Females were significantly shorter than males ($\underline{F}$ (1,44) = 203.647, $\underline{p} < 0.05$) No significant effect for housing or housing X gender interaction | | |

**Figures**

Figure 1. Non-Enriched Housing



Figure 2. Enriched Housing

Figure 3. Super-Enriched Housing

Figure 4. Individual Subject Activity Data Sheet

Date _____

Home Cage Activity (3 Minute Observations)

**Subject:_____**            **Condition: _____**            **Rater Initials: _____**

Circle a number between 1 and 7.

Horizontal Movement

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| /------------/----------/----------/----------/----------/------------/ |
| None | Almost No Activity | Low Activity | Some Activity | Moderate Activity | Intermittent High Activity | Continuous High Activity |

Vertical Movement

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| /------------/----------/----------/----------/----------/------------/ |
| None | Almost No Activity | Low Activity | Some Activity | Moderate Activity | Intermittent High Activity | Continuous High Activity |

Level of In-Place Movement

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| /------------/----------/----------/----------/----------/------------/ |
| None | Almost No Activity | Low Activity | Some Activity | Moderate Activity | Intermittent High Activity | Continuous High Activity |

Level Locomotor Movement

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| /------------/----------/----------/----------/----------/------------/ |
| None | Almost No Activity | Low Activity | Some Activity | Moderate Activity | Intermittent High Activty | Continuous High Activity |

Center Time Movement

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| /------------/----------/----------/----------/----------/------------/ |
| None | Almost No Center Activity | Low | Evenly Split | Moderate Activity | High Center Activity | All Center |

Description/Comments:_____
_____
_____
_____
_____

Figure 5. Group Activity Data Sheet

Date _____

Time _____

**Home Cage Activity (1 Minute Observations)**

Condition: _____          Rater Initials: _____          Experimental Day _____

Circle a number between 1 and 7.

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Number of Animals Moving | None | 1-2 | 3-4 | 5-6 | 7-8 | 9-10 | 11-12 |

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Amount of Activity for Majority of Group Members | None | Almost No Activity | Low Activity | Some Activity | Moderate Activity | Intermittent High Activty | Continuous High Activity |

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Level of Activity | None | Almost No Effort | Low Effort | Some Effort | Moderate Effort | Intermittent High Effort | Continuous High Effort |

Indicate the type of activity and the number of animals engaged in each type of activity:

w/Physical Object          Social Interaction          Combined P & S          Alone
_____          _____          _____          _____

Description/Comments:_____

_____

_____

_____

_____

Figure 6. Open Field Chamber

Figure 7a. Experiment I Body Weights (bar)


Figure 7b. Experiment I Body Weights (line)


Figure 7c. Experiment I Body Weight by Phase


Figure 8. Experiment I Body Mass Index

Figure 9. Experiment I Lee Index



Figure 10a. Experiment I Average Number of Grams Consumed Daily by Experimental Phase



Figure 10b. Experiment I Average Number of Calories Consumed Daily by Experimental Phase



Figure 10c. Experiment I Phase B Average Grams Breakdown

Figure 10d. Experiment I Phase B Average Calories Breakdown



Figure11a. Experiment I Median Score for Number of Animals Moving by Experimental Phase



Figure 11b. Experiment I Median Score for Amount of Physical Activity



Figure 11c. Experiment I Median Score for Level of Physical Activity

Figure 12a. Experiment I Horizontal Activity Separated by Experimental Phase



Figure 12b. Experiment I Vertical Activity Separated by Experimental Phase



Figure 12c. Experiment I Average Horizontal Activity Separated by Experimental Phase



Figure 12d. Experiment I Average Vertical Activity Separated by Experimental Phase

## Figure12e. Experiment I Averaged Within Session Horizontal Activity From All OF Measurements (HACT from all 6 OF trials averaged
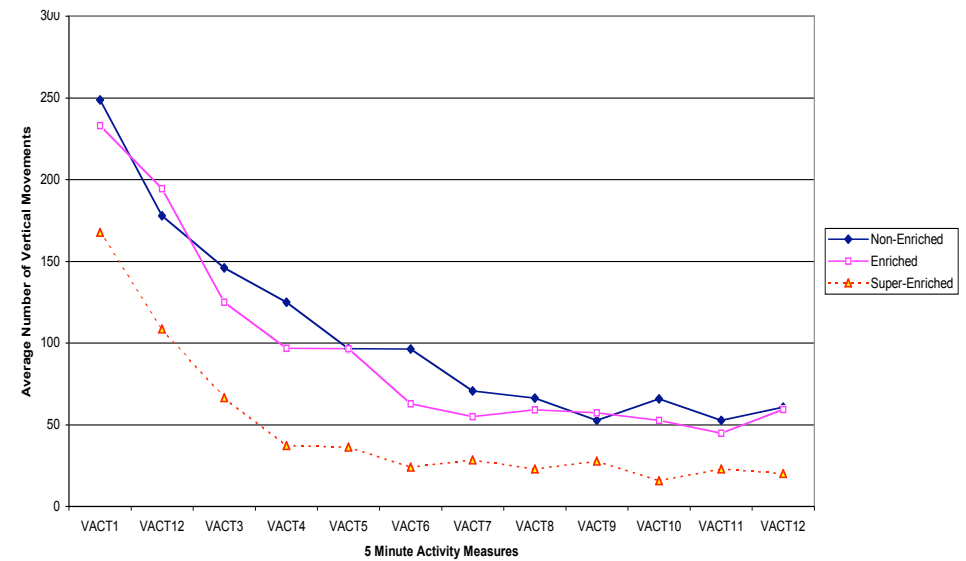


## Figure12f. Experiment I Averaged Within Session Vertical Activity From All OF Measurements (VACT from all 6 OF trials averaged together)



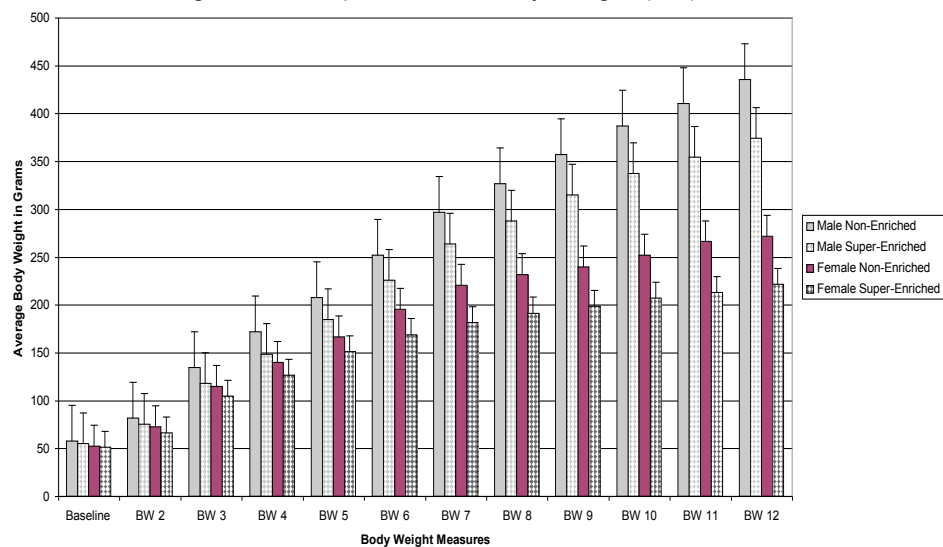## Figure 13a. Experiment II Body Weights (bar)
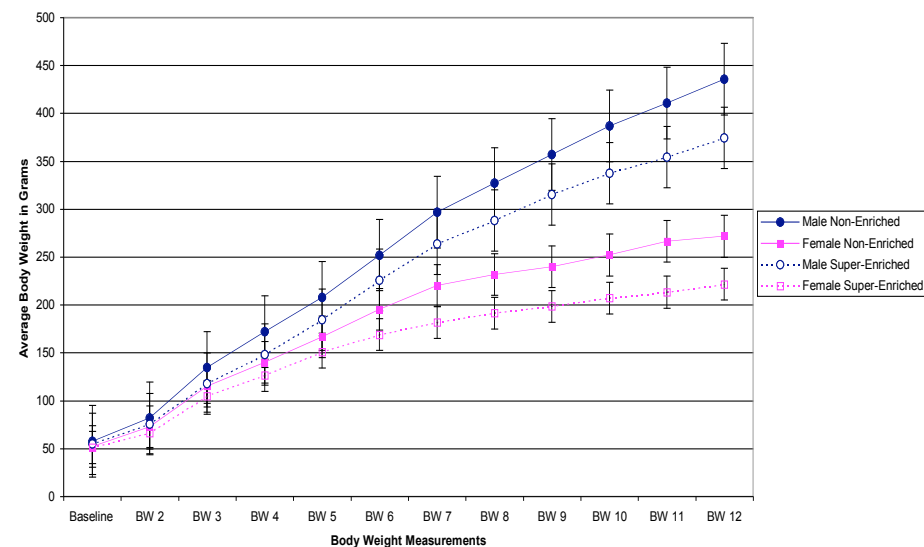


## Figure 13b. Experiment II Body Weights (line)

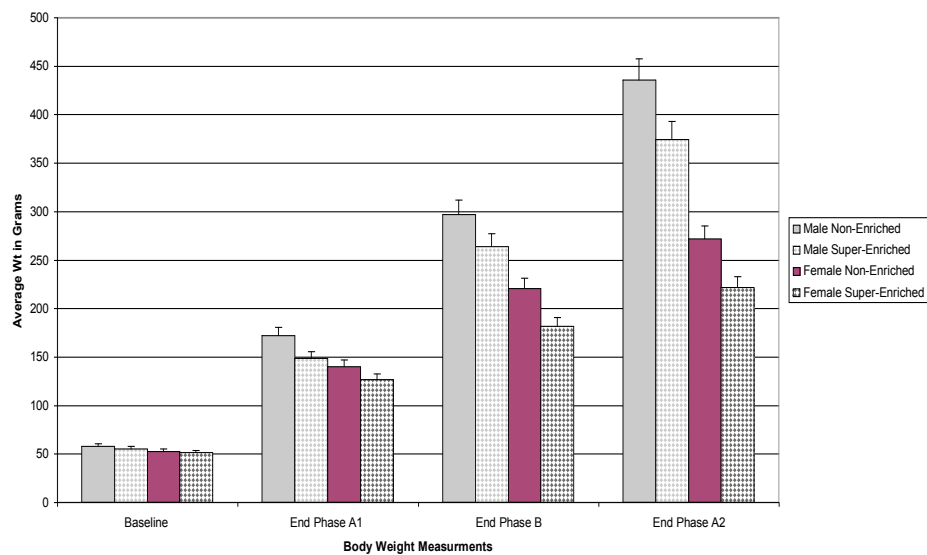## Figure13b. Experiment II Body Weight by Experimental Phase (bar)



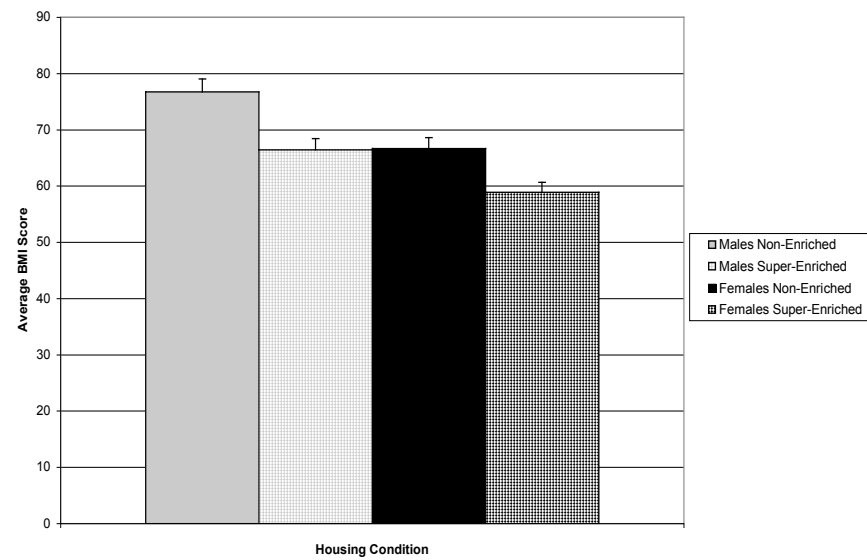## Figure14. Experiment II Body Mass Index



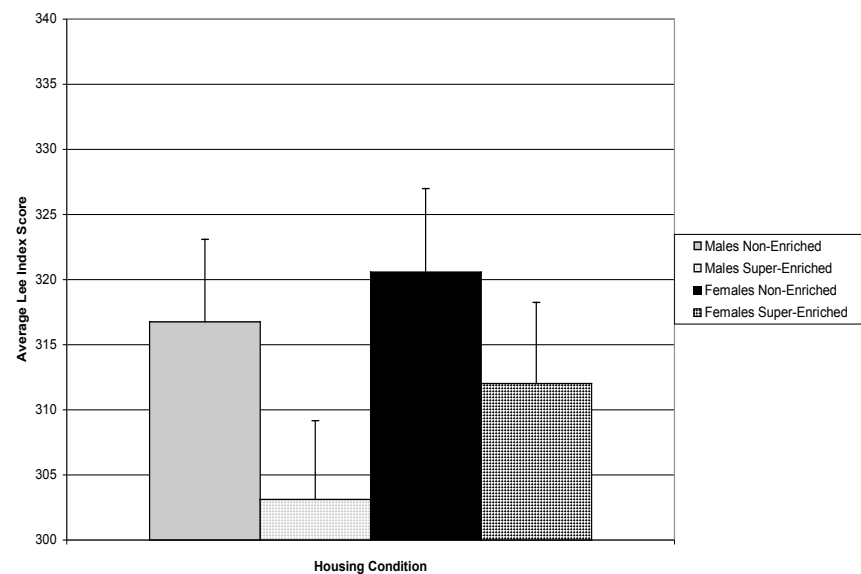## Figure15. Experiment II Lee Index



## Figure16a. Experiment II Average Number of Grams Consumed Daily by Experimental Phase

187
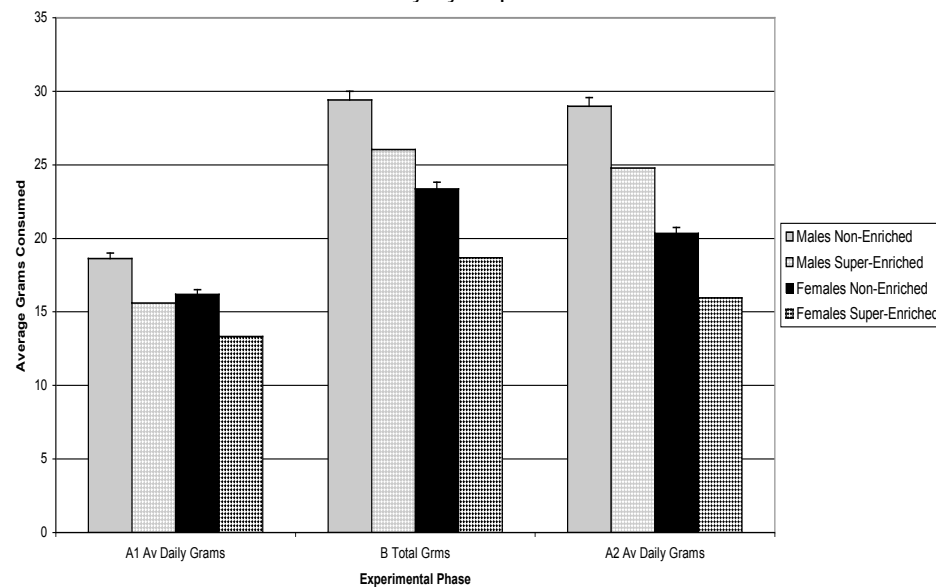


Figure16b. Experiment II Average Number of Calories Consumed Daily by Experimental Phase
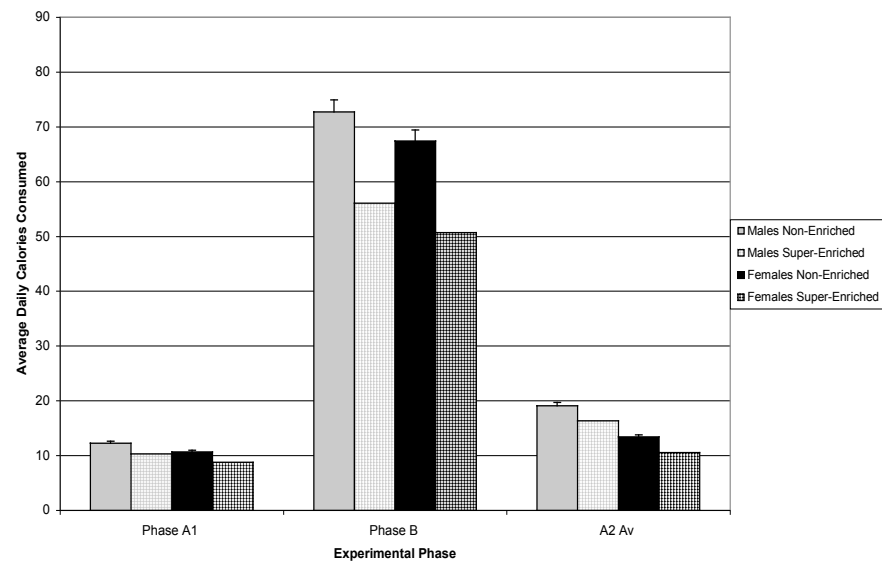


Figure16c. Experiment II Phase B Average Grams Breakdown



Figure 16d. Experiment II Phase B Average Calories Breakdown



Figure17a. Experiment II Median Score for Number of Animals Moving by Experimental Phase

Figure 17b. Experiment II Median Score for Amount of
Physical Activity by Experimental Phase



Figure 17c. Experiment II Median Score for Level of
Physical Activity by Experimental Phase



Figure 18a. Experiment II Horizontal Activity
Separated by Experimental Phase



Figure 18b. Experiment II Averaged Horizontal Activity
Separated by Experimental Phase

Figure 18c. Experiment II Vertical Activity Separated by Experimental Phase



Figure 18d. Experiment II Averaged Vertical Activity Separated by Experimental Phase



Figure 18e. Experiment II Averaged Within Session Horizontal Activity From All OF Measurements (HACT from all 6 OF trials averaged together)



Figure 18f. Experiment II Averaged Within Session Vertical Activity From All OF Measurements (VACT from all 6 OF trials averaged together)

Figure 19a. Experiment III Body Weight (bar)



Figure 19b. Experiment III Body Weight (line)



Figure 19c. Experiment III End of Experimental Phase Weight Comparison



Figure 20. Experiment III BMI Index

Figure 21. Experiment III Lee Index



Figure 22a. Experiment III Average Number of Grams Consumed Daily by Experimental Phase
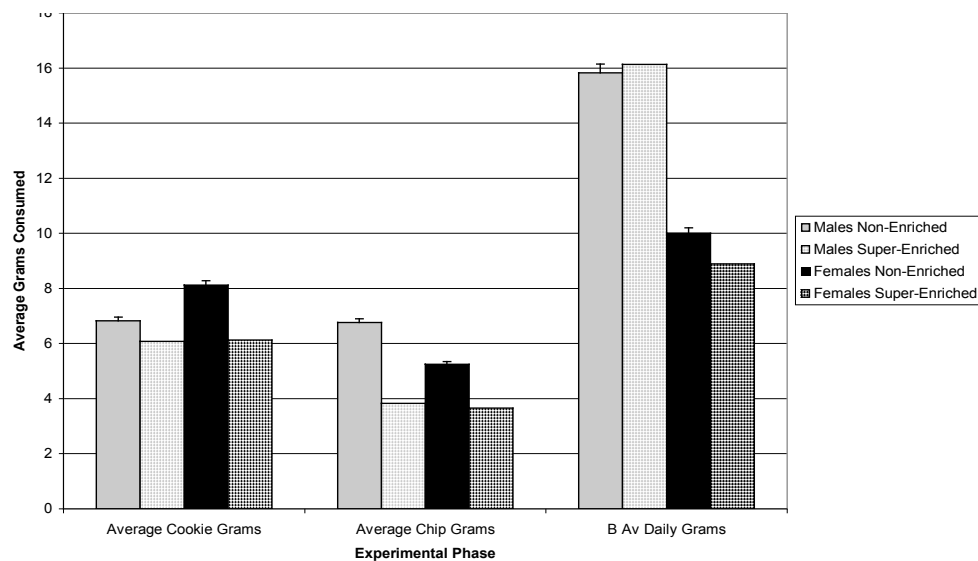


Figure 22b. Experiment III Average Number of Calories Consumed Daily by Experimental Phase



Figure 22c. Experiment III Phase B Average Grams Breakdown

Figure 22d. Experiment III Phase B Average Calories Breakdown



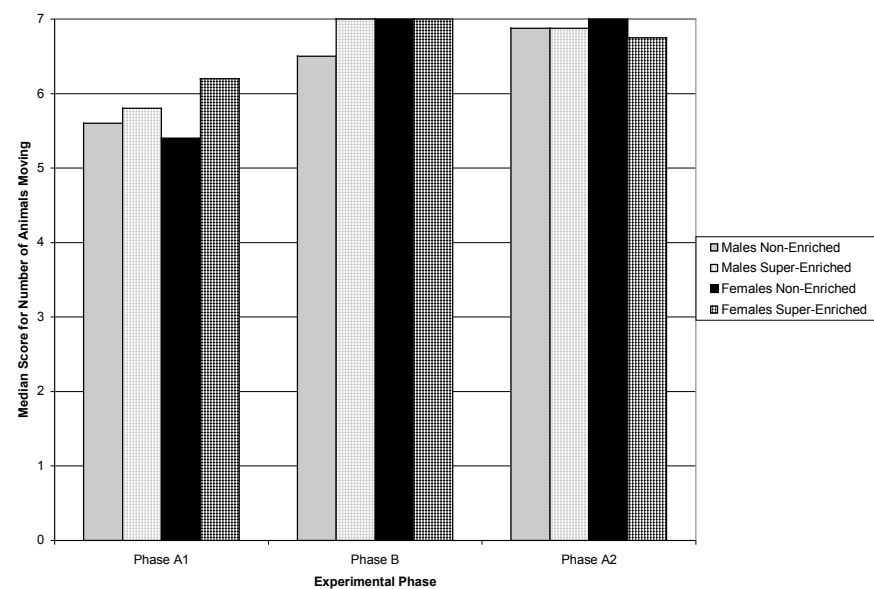Figure 23a. Experiment III Median Score for Number of Animals Moving by Experimental Phase



Figure 23b. Experiment III Median Score for Amount of Physical Activity by Experimental Phase
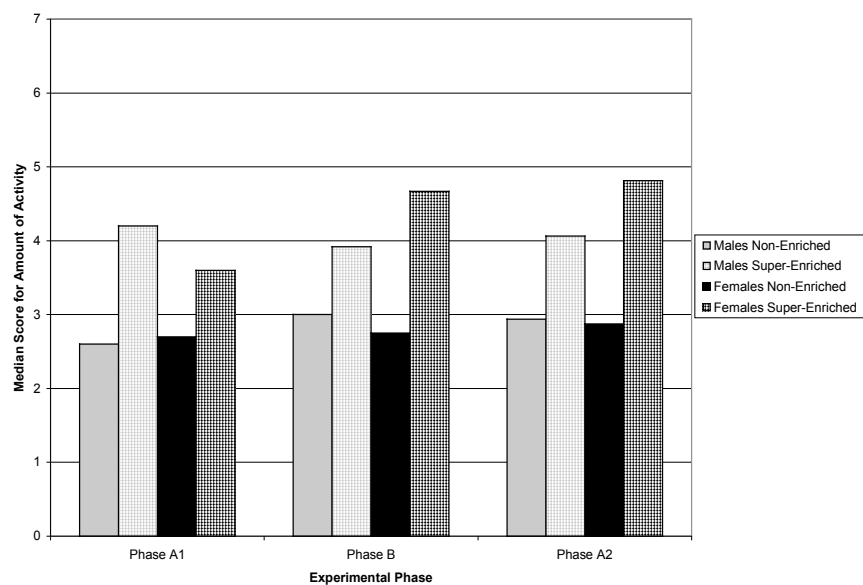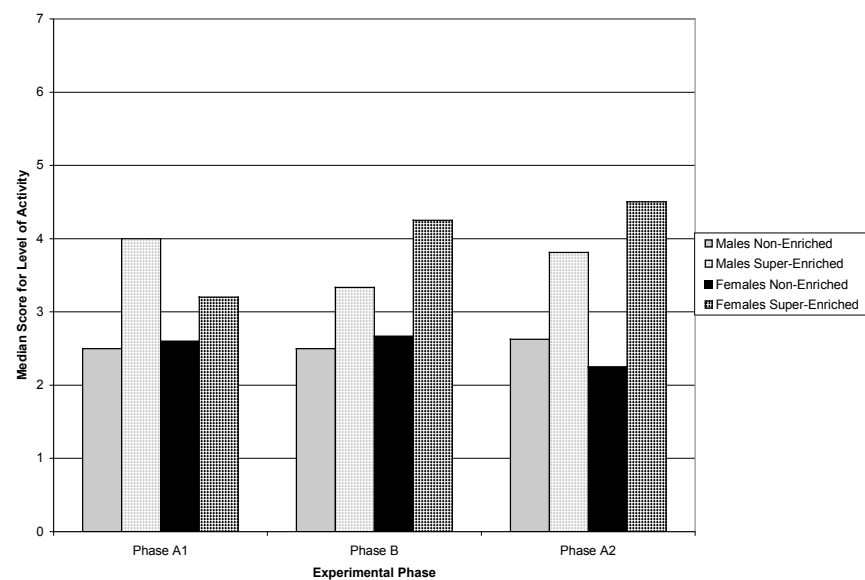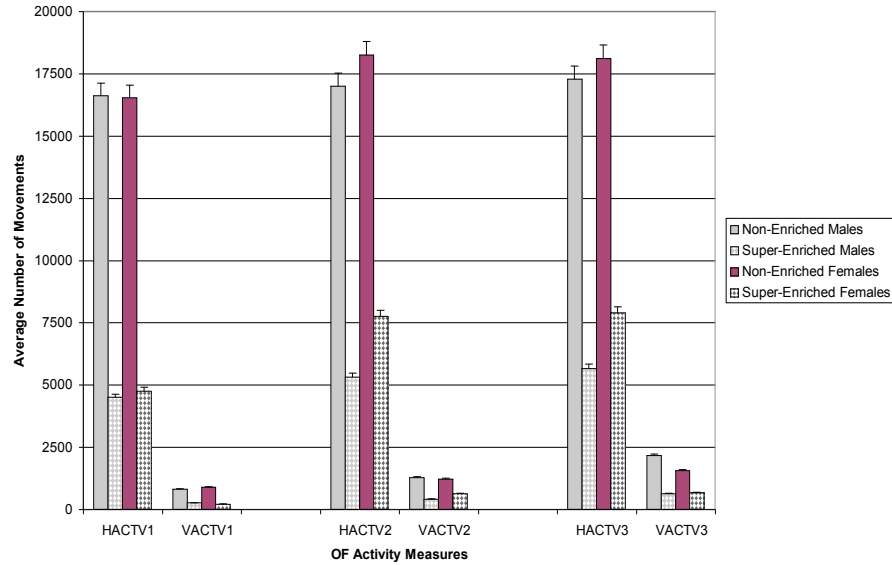


Figure 23c. Experiment III Median Score for Level of Physical Activity by Experimental Phase

24a. Average Total Horizontal Activity Separated by Experimental Phase



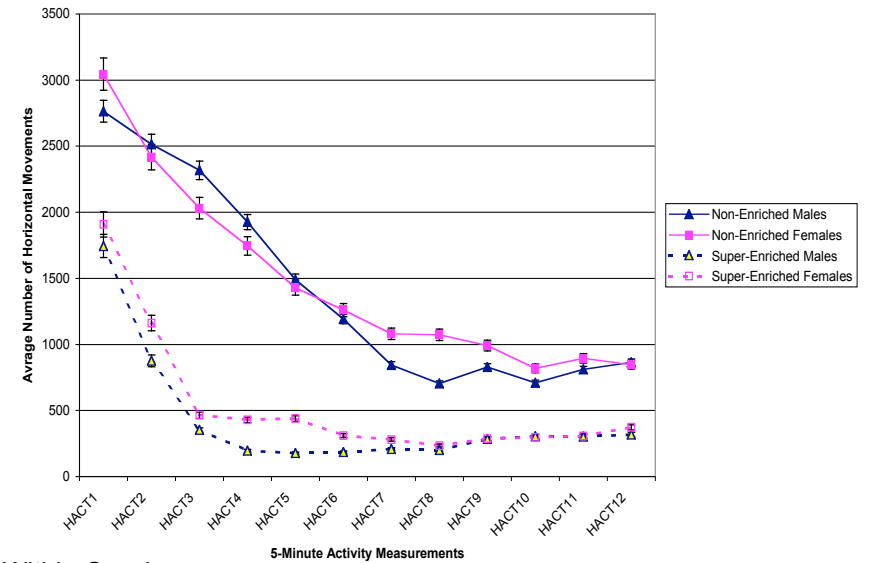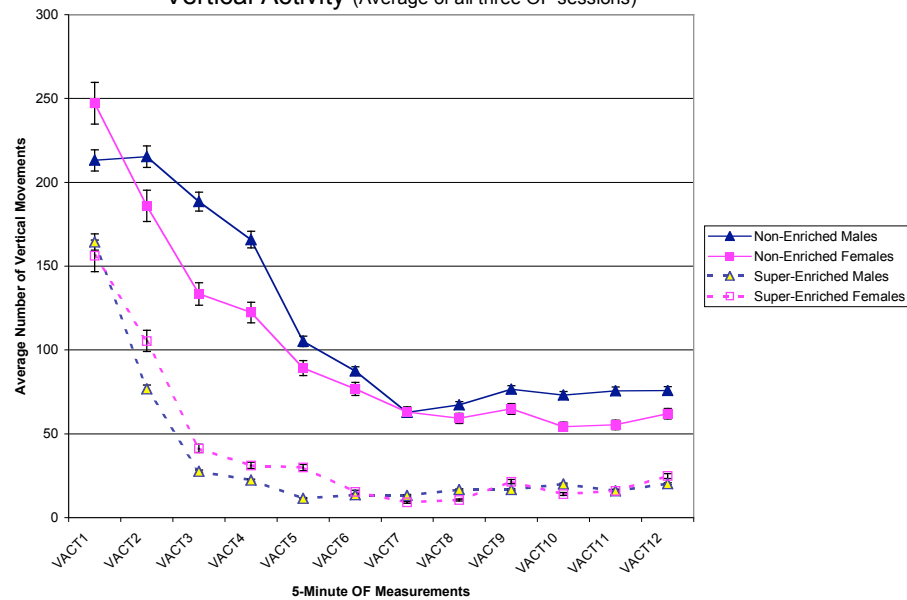24b. Experiment III Average Within-Session Horizontal Activity (Average of all three OF sessions)



Figure 24c. Experiment III Average Within-Session Vertical Activity (Average of all three OF sessions)

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